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RESEARCH PAPER

Instability in Theophylline and Carbamazepine Hydrate Tablets: Cocrystal Formation Due to Release of Lattice Water

Kapildev K. Arora • Seema Thakral • Raj Suryanarayanan

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ABSTRACT

Purpose To demonstrate two sequential solid-state reactions in intact tablets: dehydration of active pharmaceutical ingredient (API), and cocrystal formation between the anhydrous API and a second formulation component mediated by the released water. To evaluate the implication of this *in situ* phase transformation on the tablet dissolution behavior.

Methods Tablets containing theophylline monohydrate (TPM) and anhydrous citric acid (CA) were stored at 40°C in sealed polyester pouches and the relative humidity in the headspace above the tablet was continuously measured. Dehydration to anhydrous theophylline (TPA) and the product appearance (TPA-CA cocrystal) were simultaneously monitored by powder X-ray diffractometry. Carbamazepine dihydrate and nicotinamide formed the second model system.

Results The water of crystallization released by TPM dehydration was followed first by deliquescence of citric acid, evident from the headspace relative humidity (~ 68%; 40°C), and then the formation of TPA-CA cocrystal in intact tablets. The noncovalent synthesis resulted in a pronounced decrease in the dissolution rate of theophylline from the tablets. Similarly, the water released by dehydration of carbamazepine dihydrate caused the cocrystallization reaction between anhydrous carbamazepine and nicotinamide.

Conclusions The water released by API dehydration mediated cocrystal formation in intact tablets and affected dissolution behavior.

KEY WORDS carbamazepine dihydrate · cocrystal · dehydration · dissolution · phase transformation · theophylline monohydrate

INTRODUCTION

A significant fraction of active pharmaceutical ingredients (API), are capable of existing as hydrates (~30% of the compounds listed in the European Pharmacopoeia) (1). Hydrates are molecular complexes wherein water is incorporated, usually stoichiometrically, in the crystal lattice (2–4). Morris and Rodriguez-Hornedo (5) have classified them into: (1) channel hydrates (6–13) wherein water molecules interact with each other to form tunnels within the crystal lattice, (2) isolated site hydrates (14) in which water molecules are not directly hydrogen bonded to each other, and (3) metal ion associated hydrates (15) where water molecules form strong interactions with transition metals or alkali metals.

The selection of an API as a hydrate, for solid dosage form development, is typically a consequence of its desired attributes or superiority over the corresponding anhydrous phase. If a drug, in a defined state of hydration, is stable over a wide water vapor pressure range (at room temperature), formulation of the hydrate is likely to be prudent. Cephalexin, azithromycin and ampicillin are marketed as a monohydrate, dihydrate and trihydrate respectively (16–18). In spite of their perceived physical stability, dehydration of hydrates has been reported, both during pharmaceutical processing and final product storage (19). Theophylline monohydrate granules, when dried at 45°C under ambient pressure, yielded a substantially crystalline anhydrate (20). The physical form of the product phase can also be influenced by the conditions of dehydration. At ambient pressure, dehydration of ampicillin trihydrate resulted in the formation of X-ray amorphous anhydrous ampicillin, while at elevated pressures, a crystalline anhydrate was obtained (21). Mechanical stress, for example

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K. K. Arora · S. Thakral · R. Suryanarayanan (✉)
Department of Pharmaceutics, University of Minnesota
Minneapolis, Minnesota 55455, USA
e-mail: surya001@umn.edu

K. K. Arora
Pfizer Global Research & Development, Groton, Connecticut 06340, USA

Present Address:
S. Thakral
GVM College of Pharmacy Murthal Road
Sonepat, Haryana 131001, India

by milling, can also result in loss of lattice water at relatively low temperatures as has been observed in cefixime trihydrate (22).

A potentially much more serious issue is the hydrate→anhydrate transition, not during product manufacture, but in the final dosage form (typically tablets). Recently, such a transition was observed in thiamine HCl hydrate tablets (23). The liberated water can facilitate physical and chemical transformations of the formulation components. There is also potential for the liberated water to be sorbed by a superdisintegrant and alter its functionality (24,25).

Lactose and dibasic calcium phosphate, two widely used excipients, are also capable of existing as hydrates. While the former occurs as a monohydrate (26), the latter is a dihydrate (dibasic calcium phosphate dihydrate; DCPD) containing 20.9% w/w water (27–29). In solid dosage forms, decomposition of aspirin was attributed to the water released by the dehydration of DCPD (30,31). We recently reported cocrystal formation between carbamazepine and nicotinamide in intact tablets which was mediated by the water released due to dehydration of DCPD (32). While this can be a potentially serious issue in formulations, it is instructive to recognize that the dehydration of an excipient was responsible for this unintended cocrystal formation. The problem can be avoided by judicious excipient selection. When the API used in the formulation exists as a hydrate, it is assumed that the water in the lattice will not be available for interaction with the formulation components. However, the dehydration propensity of pharmaceutical hydrates warrants careful consideration particularly at elevated temperatures and reduced water vapor pressures (33–36). If the released water can mediate cocrystal formation, this has the potential to cause profound changes in product performance. In this report, we have documented two sequential reactions in intact tablets: (a) dehydration of API, and (b) cocrystal formation between the anhydrous API and a second formulation component mediated by the released water. We used scanning electron microscopy, powder X-ray diffractometry and continuous monitoring of water vapor pressure (in the tablet headspace) to comprehensively monitor these phase transformations and characterize the final product. Finally, the implication of these *in situ* phase transitions on the final product performance was evaluated. The dissolution behavior of the dosage form was used as a measure of the product performance.

MATERIALS AND METHODS

Carbamazepine, nicotinamide, theophylline and citric acid (anhydrous) were purchased from Sigma-Aldrich, St. Louis, MO (molecular structures in Scheme 1). Carbamazepine dihydrate ($C_{15}H_{12}N_2O \cdot 2H_2O$) and theophylline monohydrate ($C_7H_8N_4O_2 \cdot H_2O$) were prepared from the corresponding anhydrous phases using the reported methods (8,36,37). The first model system consisted of theophylline monohydrate (TPM) and anhydrous citric acid (CA). TPM is known to form a cocrystal with citric acid (38). TPM dehydrates under ambient conditions (25°C; RH< 75%) (36,39) and the anhydrate is known to form cocrystals with citric acid in the presence of water vapor (38).

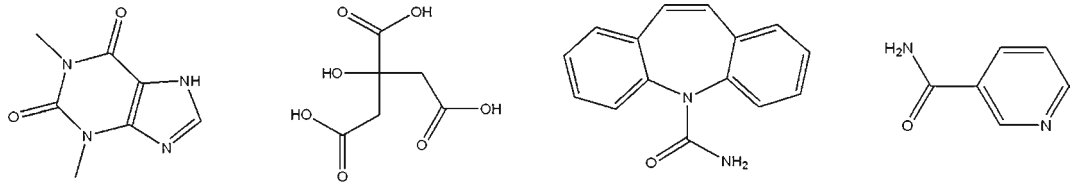
Each tablet containing TPM (106.7 mg, 0.5 mM) and CA (103.4 mg, 0.5 mM) was compressed at room temperature (~ 23°C) under controlled water vapor pressure (relative humidity, RH = 50 ± 5%). The powder mixture was filled in a circular stainless steel holder (10 mm diameter; flat face beveled edge) and compressed in a hydraulic press (Carver Model C Laboratory press, Menomonee Falls, WI) to a pressure of ~115 MPa and held for 1 min. TPM was stable during the time course of tablet preparation. The transition of TPM to theophylline anhydrate (TPA) occurred only at RH≤45% at RT (39). The tablets were immediately sealed in polyester (Mylar®) pouches and stored at 40°C. At selected time points, tablets were subjected to powder X-ray diffractometry and scanning electron microscopy. They were discarded after analyses.

There were two controls. (i) Tablets consisting of an equimolar mixture of anhydrous theophylline (TPA, 97.0 mg, 0.5 mM) and CA (103.4 mg, 0.5 mM) were compressed at room temperature under controlled water vapor pressure (45–55% RH). (ii) Tablets consisting of an equimolar mixture of TPM (106.7 mg, 0.5 mM) and CA (103.4 mg, 0.5 mM) were compressed at room temperature, and stored at RT at ~70% RH.

Carbamazepine dihydrate (CBZD) and nicotinamide (NMA) formed the second model system. Only the experimental details that are different from that of the theophylline-citric acid system are presented here. Each test tablet containing CBZD (142.1 mg, 0.5 mM) and NMA (68.2 mg, 0.5 mM) was compressed at room temperature (~ 23°C) under controlled water vapor pressure (RH = 50 ± 5%). Control tablets consisting of an equimolar mixture of anhydrous carbamazepine (CBZA, 132.0 mg, 0.5 mM) and NMA (68.2 mg, 0.5 mM) were compressed at room temperature under controlled water vapor pressure (50 ± 5% RH).

Powder X-ray Diffractometry

Intact tablets were placed in specially fabricated aluminum holders and exposed, at room temperature, to CuKα radiation (1.54 Å; 45 kV×40 mA) in a powder X-ray diffractometer (Bruker D5005). XRD patterns were typically obtained from 5 to 40° 2θ, with a step size of 0.05° 2θ. In selected instances, the patterns were also obtained over the angular range of 6 to 22° 2θ, with a step size of 0.01° 2θ. In both the cases, the counts were accumulated for 1 s at each step. Data analyses were performed using commercially



Scheme I Molecular structures of parent compounds used in the study.

available software (JADE, Materials Data, Livermore, California).

Scanning Electron Microscopy

The tablets were placed on aluminum stubs using a double-sided carbon tape, coated with platinum (50 Å), and viewed in a scanning electron microscope (Jeol 6500 F microscope Hitachi, Japan).

Headspace Humidity Measurement

The relative humidity and temperature in the headspace above the tablet, placed in a sealed Mylar® pouch, was monitored. This was accomplished by sealing one end of a digital humidity sensor (EK- H4, Sensirion AG, Switzerland) into the Mylar® pouch containing the tablet, while the other end of the sensor was connected through an interface to a computer (Fig. 1). This enabled us to continuously measure the headspace RH and temperature in the sealed pouches.

Tablet Dissolution

The United States Pharmacopeia Type 2 dissolution testing apparatus (Varian 705 DS, Varian Inc., Palo Alto, California) with a paddle speed of 50 rpm was used. The dissolution medium was deionized water (900 mL) maintained at 37 ± 0.5°C. Aliquots (5 mL) were withdrawn at predetermined time intervals, filtered (0.45 μm Millipore filter) and the absorbance at 272 nm was measured using a UV/Vis spectrophotometer (Cary Bio 100 spectrophotometer, Varian Inc., Walnut Creek, California). Control (TPA + CA) and test (TPM + CA) tablets containing 2% w/w sodium starch glycolate (tablet disintegrant) were compressed and stored at 40°C in sealed Mylar® pouches.

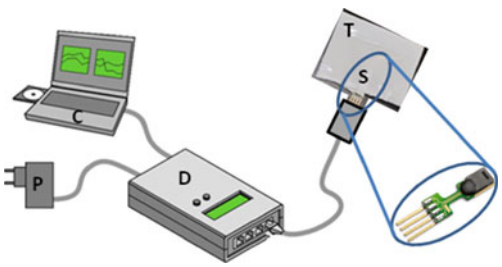


Fig. 1 Schematic representation of headspace humidity measurement assembly. S - humidity sensor, D – digital recorder, P – power cord, C – computer for data storage, T – Mylar® pouch containing tablet and sensor. An expanded view of the sensor is also provided. The Mylar® pouch containing tablet and sensor was stored at 40°C and the relative humidity data was continuously recorded and stored on the computer connected through a digital recorder. Parts of the diagram have been modified from www.sensirion.com.

We obtained the UV spectra of aqueous solutions of: (i) TPA (0.5 mM), (ii) TPA-CA cocrystal (0.5 mM), and (iii) physical mixture of TPA and CA (0.5 mM each) over the wavelength range of 230 – 400 nm. The absorbance values over the entire wavelength range were virtually superimposable, with the maximum at ~272 nm. These results indicated that, following dissolution in water, the cocrystals dissociated rapidly and the measured absorbance was that of theophylline with no interference from citric acid. By varying the citric acid concentration in aqueous solution, we also confirmed that over the entire concentration range of interest to us (0 to 0.6 mM), citric acid did not contribute to the absorbance of theophylline.

RESULTS AND DISCUSSION

Theophylline System

Two theophylline-citric acid cocrystal systems are known: theophylline-citric acid (TPA-CA cocrystal, anhydrous) and theophylline-citric acid-water (cocrystal hydrate; one molecule each of TPA, CA and water) (38).

When an equimolar mixture of TPM and CA was compressed and subjected to XRD (Fig. 2), it revealed all the characteristic peaks of TPM [for example, d-spacings of 6.6 (13.4° 2θ), 7.7 (11.5° 2θ) and 9.9 Å (8.9° 2θ)] and CA [d-spacings of 4.9 (18.1° 2θ) and 5.3 Å (16.6° 2θ)]. These tablets were stored at 40°C in Mylar® pouches, and after 2 and 4 h of storage, a characteristic peak of TPA was observed [for example with d-spacing of 7.0 Å (12.7° 2θ)] suggesting dehydration of TPM (Fig. 2a, red box). However cocrystal formation was not immediately evident. Peaks unique to TPA-CA cocrystal (anhydrous) (38), for example with d-spacings of 5.1 (17.5° 2θ) and 3.4 Å (26.0° 2θ), were observed after storage of the tablets for 19 h (Fig. 2a, purple boxes). The intensity of these peaks

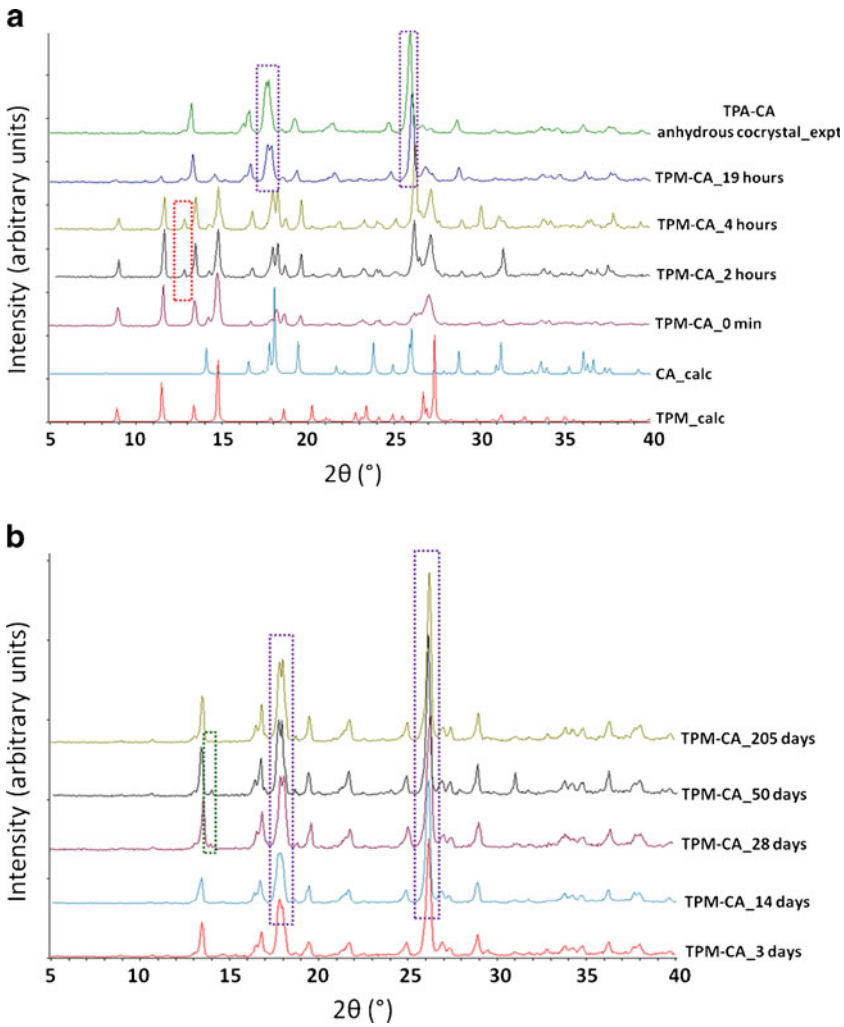


Fig. 2 X-ray diffraction (XRD) patterns of test tablets containing an equimolar mixture of TPM and CA as a function of time: (**a**) up to 19 h, and (**b**) 3 to 205 days of storage. The tablets were stored at 40°C in sealed Mylar® pouches. The *red box* shows a characteristic peak of TPA, the *purple boxes* show some characteristic peaks of TPA-CA cocrystal (anhydrous) and the *green box* shows a characteristic peak of TPA-CA monohydrate cocrystal. The XRD patterns of TPM, CA and TPA-CA anhydrous cocrystal are also provided. The calculated patterns were obtained from single crystal data from Cambridge Structural Database (40,41).

increased slightly as a function of storage time. These results suggest that transition of TPM to TPA was *followed* by TPA-CA cocrystal formation (Fig. 2a and b). Following 72 h of storage, the characteristic peaks of the reactants - TPM (8.9, 11.5 and 13.4° 2θ) and CA (16.6 and 18.1° 2θ) disappeared completely. The relative humidity in the tablet headspace was observed to be ~68% (at the storage temperature of 40°C) for the first ~38 h after which the RH gradually increased to ~80% in ~6 h (Fig. 3). Thereafter the relative humidity value remained approximately constant. Thus, in spite of the high water activity in the tablet headspace, the TPA-CA anhydrous cocrystal appeared to be the preferred phase.

These results strongly suggest that cocrystal formation was mediated by the water released by dehydration of TPM. Jayasankar *et al.* elucidated the mechanism of water mediated cocrystal formation using many model systems of API and cocrystal coformer (42). The APIs selected by them, including carbamazepine, theophylline and caffeine have the propensity to form hydrates. The theophylline - dicarboxylic acid (oxalic, maleic, glutaric or malonic acid) system is of particular relevance to this discussion. In their investigations, when mixtures of anhydrous theophylline, coformer and deliquescent additive were stored above the deliquescence RH of the dry mixtures, spontaneous cocrystal formation was observed between theophylline and coformer. They explained the deliquescence-induced

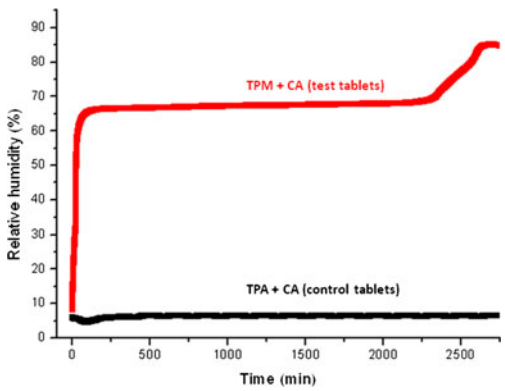


Fig. 3 Comparison of headspace relative humidity (RH) of TPM + CA (test) and TPA + CA (control) tablets stored at 40°C in sealed Mylar® pouches containing a RH sensor.

cocrystal formation in three steps: water sorption, deliquescence and cocrystal formation.

In our system, the API existed as a hydrate (theophylline monohydrate), and the tablets were stored in sealed Mylar® pouches. It is instructive to consider the sample geometry here. The total 'available' volume (tablet + headspace) in the sealed Mylar® pouch was ~0.67 cm^3 and the tablet volume was 0.16 cm^3. Therefore the headspace constituted ~0.5 cm^3. Following storage at 40°C, as shown in Fig. 3, the headspace RH was initially 68%, then increased to 80% (in less than 2 days) and was approximately constant thereafter (data not shown). This increase in RH is attributed to the water released by the dehydration of TPM. After complete dehydration of TPM (this will occur in about 3 days; Fig. 2), ~9.7 mg of water will be released. However, the amount of water required to raise the RH of the headspace to ~70% (40°C) is only~ 0.02 mg (the calculations and the relevant information are provided as Supplementary Material). Therefore the rest of the water will be available for interaction with the formulation components. The critical relative humidity (or deliquescence RH, RH_0) of anhydrous citric acid has been reported to be 70% at 40°C (43). We observed the headspace RH to be ~68% during the first 38 h (Fig. 3). We believe that deliquescence of citric acid occurs in the tablet and is responsible for the constant headspace RH of ~68% during the first ~38 h (Fig. 3). The aqueous solubility of citric acid at 40°C is reported to be 9.5 M (44). While theophylline will also dissolve in the deliquesced solution, in light of its low aqueous solubility (~0.07 M at 37°C at pH 1.2) (45) its concentration is expected to be very low and will have only a small effect on the solution RH. This large concentration difference between the API and coformer will readily result in supersaturation with respect to the TPA – CA cocrystal which will then crystallize from solution (42). The RH in the headspace will remain constant as long as the liberated water is saturated with citric acid. However, as the citric acid in solution crystallizes as the TPA–CA cocrystal, eventually the liberated water will no longer be saturated with citric acid. The headspace RH then increases and reaches a value of~80%.

According to Karki *et al.* (38) the anhydrous cocrystal (TPA-CA) could be obtained by processing the anhydrous cocrystal components in the absence of water. The cocrystal hydrate was prepared by either (i) dry grinding, as long as one of the cocrystal components was a monohydrate (both theophylline and citric acid exist as a monohydrate), or (ii) when anhydrous theophylline and citric acid were ground with water (liquid assisted). Our result is different from that of Karki *et al.* and one possible explanation is the specific process and the conditions used. Karki *et al.* milled the powder mixture under ambient conditions (temperature of the reaction mixture < 35°C). We compressed the powder mixture at 50% RH (RT) so as to ensure the physical stability of TPM during tablet manufacture and stored the tablets in sealed Mylar® pouches.

In an elegant study, the phase behavior of anhydrous/hydrated cocrystals of theophylline and citric acid were elucidated by Jayasankar *et al.* (46). They generated a drug-coformer-water triangular phase diagram showing the regions of stability of the anhydrous (TPA-CA) and hydrate (TPA-CA-water) cocrystals. As one of the system components undergoes deliquescence, it was possible to predict the transition pathways as well as the crystallizing phase using the phase diagram. When deliquescence was initiated, the anhydrous cocrystal was the stable phase (least soluble). However, when the concentration of the coformer fell below that of the anhydrous cocrystal and cocrystal hydrate eutectic, then the anhydrous cocrystal would transform to the cocrystal hydrate.

Our results indicate the preferred formation of anhydrous cocrystal and its existence during the entire course of storage (Fig. 2b). The hydrate cocrystal is characterized by unique intense peaks at 14.2, 16.8, 25.0, 27.6, and 28.8° 2θ (Cu Kα radiation) (38). Even if the cocrystal hydrate is formed (based on the single peak at 14.2° 2θ), there was no evidence of anhydrous cocrystal→hydrate cocrystal transformation during the storage time. This conclusion is based on several observations: (i) There was no pronounced increase in the intensity of the 14.2° 2θ peak. (ii) None of the other characteristic peaks of cocrystal hydrate (16.8, 25.0, 27.6 and 28.8° 2θ) were observed. (ii) There was no discernible decrease in the intensities of several characteristic peaks of TPA-CA anhydrous cocrystal (17.5 and 26.0° 2θ) between 28 and 205 days of storage.

The first set of control tablets, as mentioned earlier, contained an equimolar mixture of TPA and CA. Even after 165 days of storage, there was no measurable decrease in the intensities of the characteristic peaks of TPA and CA and no evidence of cocrystal formation (Fig. 4). Earlier, in the test tablets, we had observed cocrystal formation (Fig. 2), believed to be mediated by the water released by dehydration of TPM. The absence of the solid-state reaction in

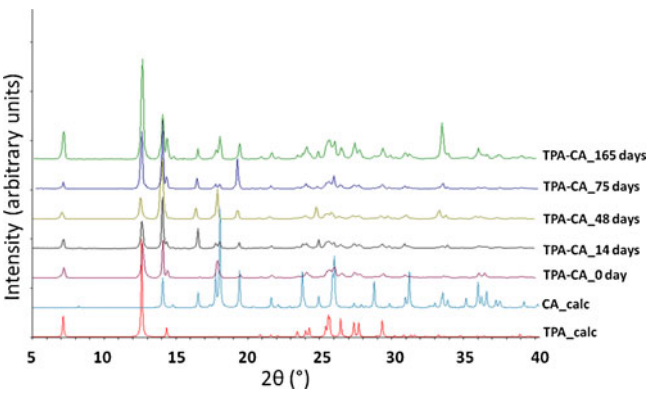


Fig. 4 XRD patterns of control tablets containing an equimolar mixture of TPA and CA as a function of time. The tablets were stored at 40°C in sealed Mylar® pouches. Cocrystal formation was not evident up to 165 days of storage. The calculated XRD patterns of TPA and CA are also provided. The calculated patterns were obtained from single crystal data from Cambridge Structural Database (40,41).

control tablets provided strong evidence of the role of water in mediating cocrystal formation.

In order to verify the role of released water on cocrystal formation in tablets, an equimolar mixture (powder) of TPM and CA was filled in the sample holder of a powder X-ray diffractometer, sealed with Kapton® tape, held at 50°C, and XRD patterns were periodically obtained. Kapton® is transparent to X-rays (47) and the tape effectively sealed the holder preventing the escape of dehydrated water. This was done to simulate the conditions in the headspace of the Mylar® pouches. In 60 min, there was the first evidence of TPA-CA anhydrous cocrystal formation (Fig. 5). There was a progressive increase in the peak intensities and the XRD pattern at 480 min (provided as a representative example), revealed intense, sharp peaks of TPA-CA anhydrous cocrystal. There was no evidence of hydrate cocrystal formation. As discussed earlier, in the tablets stored at 40°C as well, anhydrous cocrystal formation was observed but after 19 h of storage. This is not surprising in light of the lower temperature of tablet storage.

In contrast, when the equimolar mixture was held at 50°C in an open sample holder (*i.e.* without sealing the sample holder with Kapton® tape), dehydration of TPM was followed by the formation of TPA. This conclusion was based on the decrease in the intensity of the 8.9° 2θ peak of TPM followed by the appearance and increase in intensity of the 12.7° 2θ peak of TPA (Fig. 6). Interestingly, neither the anhydrous cocrystal nor cocrystal hydrate was formed during the entire experiment. These results provide further evidence of the role of water in mediating cocrystal formation in tablets.

Since the water released by the dehydration of TPM was believed to mediate the cocrystal formation, a second set of control tablets were prepared using TPM. Therefore, compositionally, these control tablets are identical to the test tablets. However, the tablets were stored under conditions at which the hydrate would be the stable phase (~70% RH; stored at RT). After 26 days of storage, there was no evidence

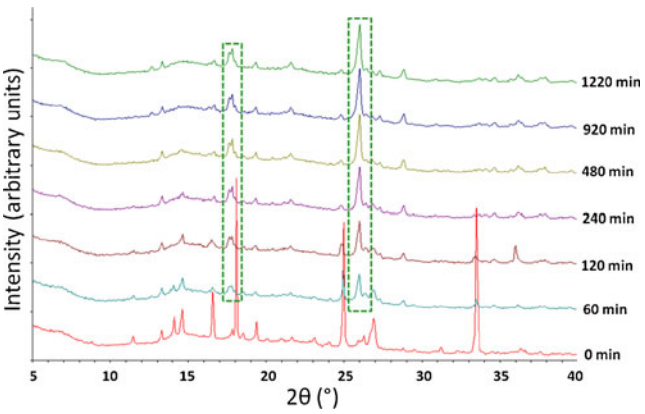


Fig. 5 XRD patterns of equimolar mixture of TPM and CA, held at 50°C. The sample was sealed with Kapton® tape to prevent the release of dehydrated water. The *green boxes* reveal the characteristic peaks of TPA-CA cocrystal (anhydrous).

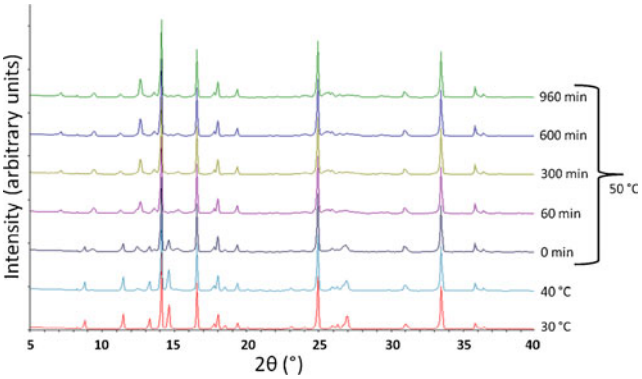


Fig. 6 XRD patterns of equimolar mixture of TPM and CA, held at 50°C. The experiment was performed in an open holder so as to enable release of water after dehydration.

of either dehydration or cocrystal formation. In other words, there was no phase transformation of TPM in these tablets. Even though one of the components was TPM, the lattice water in TPM was "immobile". This is further evidence that, in the test tablets, the water released by the dehydration of the API mediated the cocrystal formation. However, under the high storage RH, a fraction of the anhydrous citric acid transformed to citric acid monohydrate (Fig. 7, purple boxes).

Finally, we evaluated the implication of this *in situ* phase transformation on the disintegration, and more importantly, the dissolution behavior of the tablets. The disintegration time of the freshly prepared test tablet was 370 s. Following storage for 8 months at 40°C in sealed Mylar® pouches, the disintegration time increased to 680 s. Thus the phase transition was accompanied by a pronounced increase in the tablet disintegration time. The dissolution results were equally dramatic (Fig. 8). We did not have enough tablets to rigorously evaluate our systems and test whether they met the dissolution specifications for theophylline tablets in the USP. However, based on the average dissolution values, the freshly prepared test tablets (TPM + CA + sodium starch glycolate) met the

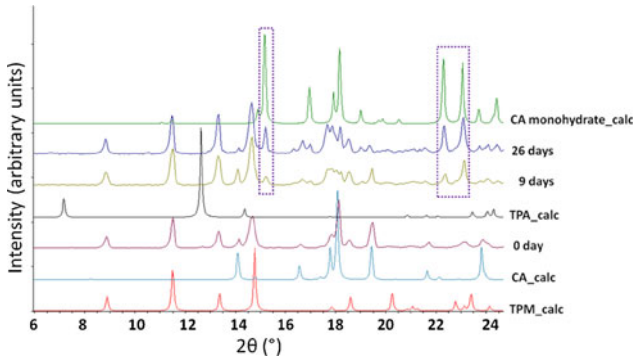


Fig. 7 XRD patterns of tablets containing an equimolar physical mixture of TPM and CA, stored at ~70% RH at room temperature. The *purple boxes* reveal appearance of the characteristic peaks of citric acid monohydrate. The calculated XRD patterns of TPM, TPA, CA, and CA monohydrate are also provided. The calculated patterns were obtained from single crystal data from Cambridge Structural Database (40,41).

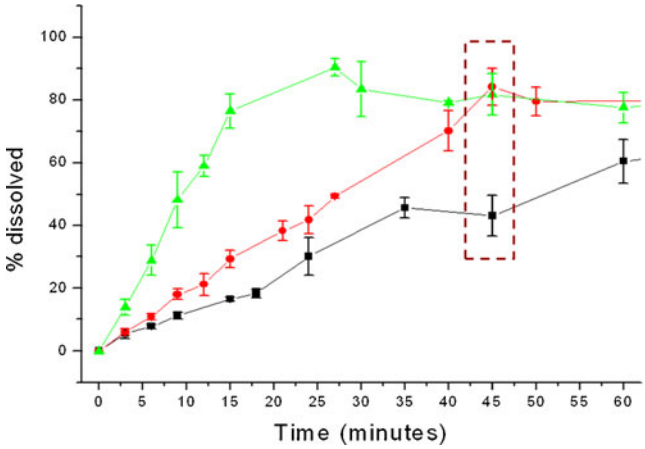


Fig. 8 Dissolution profile of theophylline in: ▲- freshly prepared TPA-CA anhydrous cocrystal tablets, ● - freshly prepared test tablets containing equimolar mixture of TPM and CA and ■ - test tablets stored at 40°C in sealed Mylar® pouches for 8 months. All the tablets contained 2% w/w sodium starch glycolate. Error bars represent standard deviations; $n=3$.

dissolution specifications for theophylline tablets in the USP (≥ 80% dissolved in 45 min). Following storage, there was a dramatic decrease in the dissolution rate and only ~ 43% of the labeled amount dissolved in 45 min. However, the freshly prepared TPA-CA cocrystal exhibited the most rapid initial dissolution rate with ~ 80% of the drug dissolving in < 30 min.

We had documented that storage at 40°C in sealed Mylar® pouches had caused cocrystal formation in the tablets (Fig. 2). However, this cocrystal formation *per se* does not appear to be responsible for the observed change in dissolution behavior. This conclusion is based on the observation that the initial dissolution rate of theophylline from the cocrystal tablet was very rapid (Fig. 8). The change in dissolution behavior upon storage can be explained by the *in situ* phase transformation affecting the functionality of sodium starch glycolate, the disintegrant in the formulation. The disintegrant exerts its action by rapidly absorbing water, swelling and as a result causing tablet disintegration. During cocrystal formation, some of the particles crystallize around the sodium starch glycolate (disintegrant) particles. In other words, the cocrystals will effectively "coat" the disintegrant particles (20). Therefore, the coating will have to dissolve first before the disintegrant particles can come in contact with the dissolution medium and exert their action. The functionality of the disintegrant is compromised and there is a pronounced delay before tablet disintegration is initiated. Thus the observed decrease in dissolution rate is not a direct consequence of cocrystal formation - cocrystallization in tablets affects the excipient functionality resulting in a decrease in dissolution rate.

Carbamazepine System

Carbamazepine dihydrate (CBZD) was selected as the second model API and nicotinamide (NMA) as the cocrystal coformer. Differential scanning calorimetry of CBZD revealed an endotherm over the temperature range of 50-80°C attributed to dehydration and vaporization of water. When heated in a thermogravimetric analyzer from RT to 100°C, a weight loss of 13.2% was observed which was in excellent agreement with the stoichiometric water content in CBZD (48). The water content determined by Karl Fischer titrimetry was also 13.2%. CBZD is stable at RT when stored at RH≥52% (49). CBZD had a much higher propensity to form a cocrystal with NMA upon co-milling (~1 min) than CBZA (~6 min) (50). This was explained by the significant role of water on the formation and stability of CBZA-NMA cocrystals (42,51).

The test and control tablets, as discussed in the Materials and Methods section, were stored at 40°C in sealed Mylar® pouches and were subjected to XRD and SEM at selected time points. After 1 day of storage, based on XRD, the dehydration of CBZD was substantially complete (Fig. 9). This conclusion

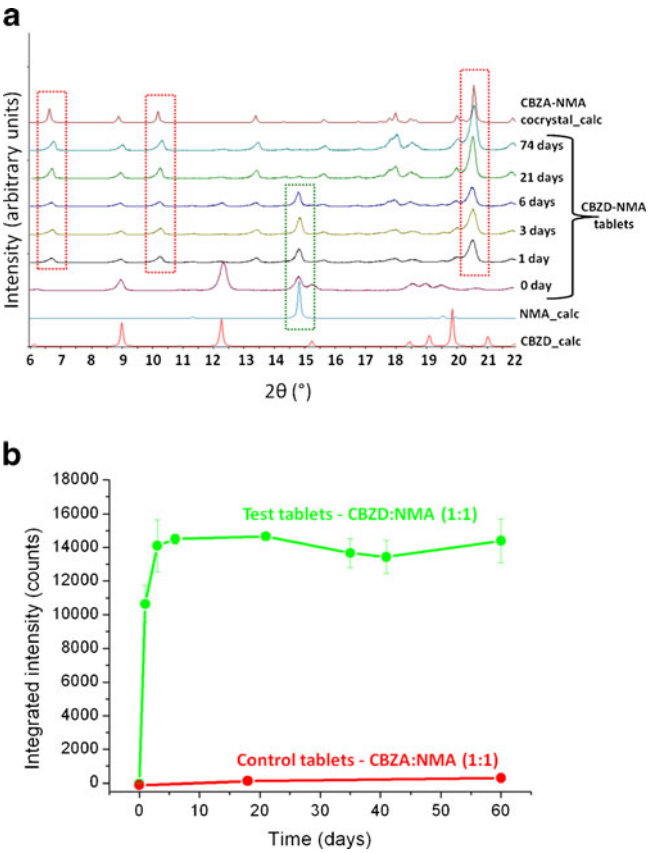


Fig. 9 (**a**) XRD patterns of test tablets containing an equimolar physical mixture of CBZD and NMA as a function of time. Data is shown up to 74 days of storage. Several characteristic peaks of CBZA-NMA cocrystal are pointed out (*red box*). NMA peak was present up to 6 days (*green box*). The calculated XRD patterns of CBZD, NMA, and CBZA-NMA cocrystal are also provided. The calculated patterns were obtained from single crystal data from Cambridge Structural Database (40,41). (**b**) Intensities of a characteristic peak of CBZA-NMA cocrystal (13.1 Å) in test (*green*) and control (*red*) tablets as a function of time. Data is shown up to 60 days of storage. (error bars represent standard deviation; $n=3$). All the tablets were stored at 40°C in sealed Mylar® pouches.

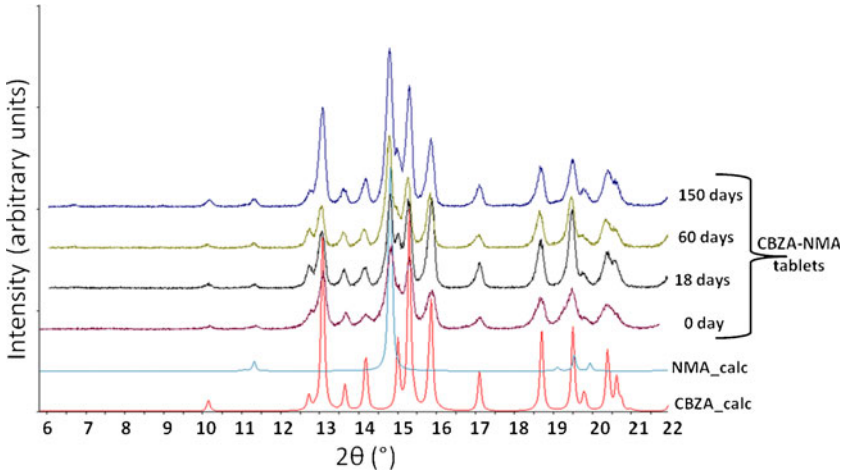


Fig. 10 XRD patterns of control tablets containing an equimolar mixture of CBZA and NMA. The tablets were stored at 40°C in sealed Mylar® pouches and periodically subjected to XRD. There was no evidence of cocrystal formation when stored up to 150 days.

was based on the very pronounced decrease in the intensity of the characteristic CBZD peak at 12.4° 2θ. The disappearance of CBZD was accompanied by the appearance of several peaks attributable to CBZA-NMA cocrystals, for example with d-spacings of 13.1 (6.7° 2θ), 8.6 (10.3° 2θ) Å and 4.3 Å (20.7° 2θ; Fig. 9a), suggesting water-mediated cocrystal formation. The second tablet component, NMA, behaved differently. Its peak intensities decreased gradually (Fig. 9a) and were not observed after six days of storage. Li *et al.* had observed that dehydration of CBZD resulted in the formation of an amorphous product (48). The mechanism of water mediated cocrystal formation was elucidated by Jayasankar *et al. (*42). Their system consisted of CBZA, NMA and sucrose, a deliquescent additive. More recently, CBZA-NMA cocrystal formation was demonstrated from an equimolar amorphous mixture of CBZA and NMA (52). Even though the NMA was crystalline in our system, cocrystal formation occurred very rapidly. Figure 9b (green profile) provides a measure of the formation kinetics. There was no evidence of cocrystal formation following storage of tablets containing an equimolar mixture of CBZA and NMA (Figs. 9b, red profile and 10). This was a strong proof of water mediated cocrystal formation in test tablets.

Earlier, in a system consisting of CBZA (120 mg, 0.50 mM) , NMA (60 mg, 0.50 mM), and DCPD (20 mg, 0.10 mM), the first evidence of cocrystal formation was observed after 22 days of storage at 40°C in sealed Mylar® pouches (32). Unlike CBZD, dehydration of DCPD occurred at a slower rate, which could explain the slower cocrystal formation kinetics.

In order to confirm the role of released water on cocrystal formation in carbamazepine test tablets, isothermal powder XRD experiments were performed. An equimolar powder mixture of CBZD and NMA was filled in the sample holder of a powder X-ray diffractometer and held isothermally at 50°C. While the CBZD rapidly transformed to CBZA, there was no evidence of cocrystal formation (data not shown). The water released by the dehydration of CBZD was able to leave the system and therefore may not have been available to mediate cocrystal formation. In a second set of experiments, an equimolar powder mixture of CBZD and NMA was filled in the sample holder of a powder X-ray diffractometer and "sealed" with Kapton® tape. The initial powder pattern, obtained at 30°C, revealed the characteristic peaks of CBZD and NMA (Fig. 11). When the sample was heated to 50°C, the CBZD dehydrated rapidly, and the

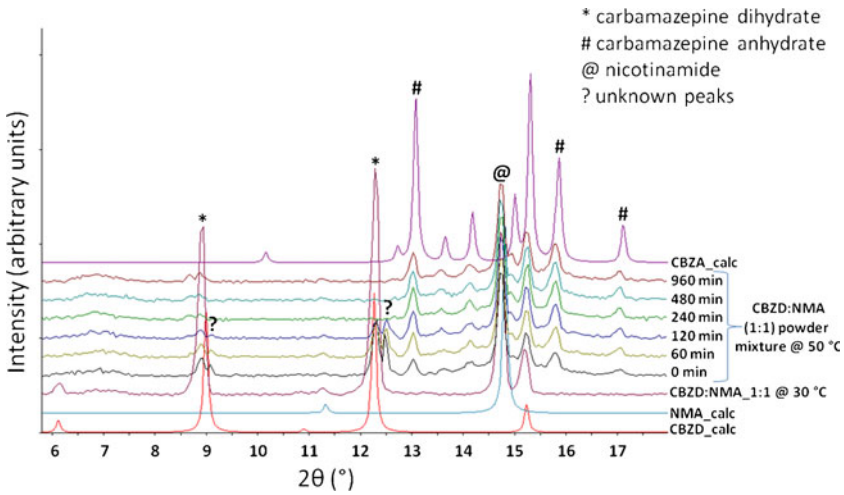


Fig. 11 XRD patterns of equimolar mixture of CBZD and NMA, held at 50°C. The sample was sealed with Kapton® tape.

characteristics peaks of carbamazepine anhydrate (form III, monoclinic, peaks at 13.1, 15.9 and 17.1° 2θ; Fig. 11) were observed. The dehydration was complete in ~120 min. However, again, there was no evidence of cocrystal formation even after 960 min, and the powder pattern revealed a mixture of CBZA and NMA. The XRD holders were weighed before and after the experiment, and there was no appreciable weight loss indicating that the water released by dehydration was available to mediate cocrystal formation. We believe that, in tablets, the dense packing of the material enables intimate contact between the reactants facilitating cocrystal formation. Moreover, the compression process is known to induce lattice disorder which in turn may increase the reactivity (20,53,54). These results suggest the potential for enhanced reactivity in tablet formulations.

The scanning electron micrographs permitted visualization of cocrystal formation on the tablet surface (Fig. 12). The test and control tablets of both theophylline and carbamazepine were stored in sealed pouches at 40°C and were subjected to SEM. In both the systems, following storage, SEM of test tablets revealed distinct changes in surface morphology, including evidence of crystal growth. Control tablets stored under identical conditions did not reveal any pronounced morphological changes suggesting no phase transformation (*i.e.* formation of cocrystals).

It is instructive to recognize that the sample preparation and SEM analyses were carried out under reduced pressure. This was of concern, since the model compounds were hydrates (TPM, CBZD) and there was the potential for dehydration. The freshly prepared control (TPA + CA; CBZA + NMA) as well as the test tablets (TPM + CA; CBZD + NMA) were analyzed by XRD before and after they were subjected to SEM. In both the test systems, after SEM imaging, the API existed in the anhydrous form. Thus there was hydrate→anhydrate conversion during SEM sample preparation and analyses. This potential experimental artifact does not affect our conclusion.

CONCLUSIONS

We believe that this is the first report of the water released by dehydration of API mediating cocrystal formation with an excipient in intact tablets. Such a transformation, by affecting the excipient functionality, has the potential to profoundly affect the final product performance. Since a significant fraction of APIs are capable of existing as hydrates (~30%) (1), the possibility of such a water-mediated cocrystal formation warrants further consideration. The solubility and dissolution of a cocrystal can be different from that of the parent API (55–61). Therefore, such *in situ* cocrystal formation, by altering the dissolution behavior, can have a pronounced effect on the final product performance. In addition, cocrystal formation can affect the mechanical properties of the dosage form (62–65). The water liberated by dehydration can also have other indirect effects. It can facilitate chemical decomposition of the formulation components (30,31). The water can be sorbed by excipients, specifically the disintegrant (66), affecting its functionality (25).

Finally, the detection and quantification of cocrystals in finished dosage forms can be analytically challenging. In this context, it is instructive to review the FDA Guidance for Analytical Procedures and Methods Validation: (67) "A

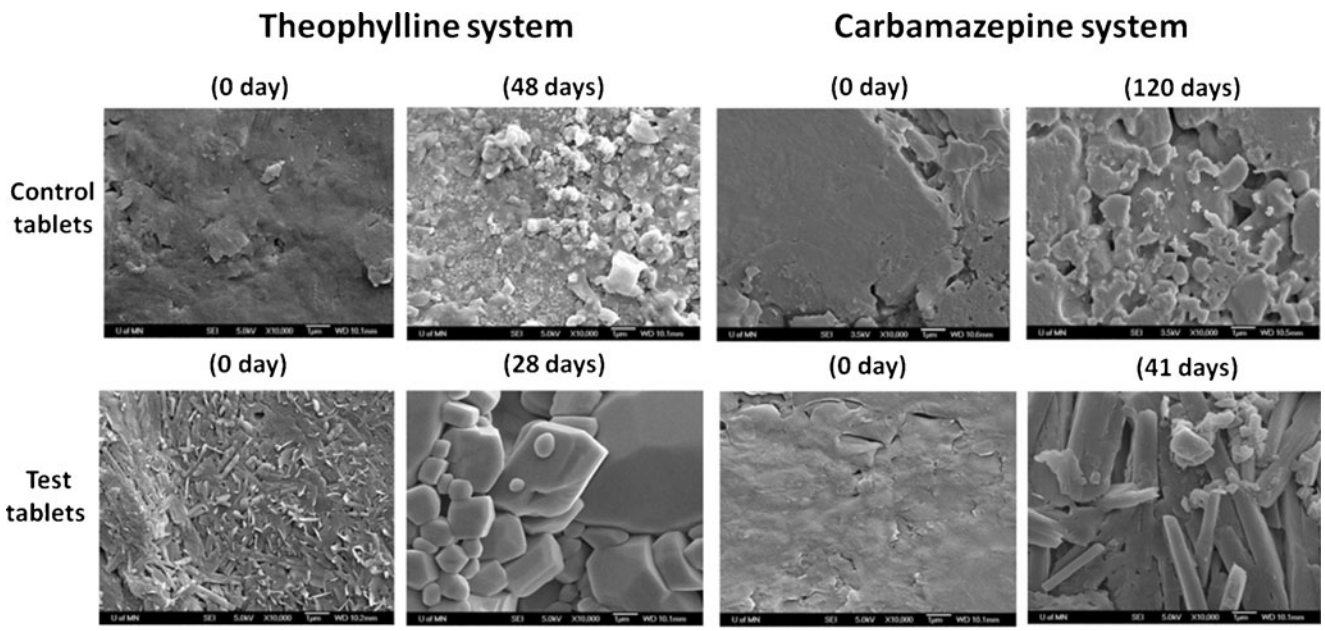


Fig. 12 Scanning electron images of (i) control tablets (*top*) – (TPA + CA) and (CBZA + NMA) and (ii) test tablets (*bottom*) – (TPM + CA) and (CBZD + NMA). The tablets were stored at 40°C in sealed Mylar® pouches.

stability-indicating assay is a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability-indicating assay accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities." Liquid chromatography finds extensive use as a stability-indicating analytical technique. Since this is a solution-based technique, the analyte should first be dissolved in an appropriate solvent. However, once in solution, a cocrystal may dissociate into the drug and cocrystal coformer. As a result, the *in situ* cocrystal formation may no longer be discerned. This problem can be circumvented by analyzing the dosage form directly using an appropriate solid-state characterization technique. Developing such a validated analytical technique, with adequate sensitivity and selectivity for cocrystal quantification in a complex and multicomponent matrix, can be a challenge.

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