Crystallization and Transformation of Acetaminophen Trihydrate

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ABSTRACT: Acetaminophen is both polymorphic and prone to the formation of a trihydrate. The recently discovered trihydrate is a lath-like crystalline form that is highly metastable with respect to conversion to the thermodynamically stable form I polymorph. While the trihydrate is physically stable in ice-cold aqueous suspension (with up to 50% propylene glycol or glycerol), conversion of trihydrate takes place at temperatures above 5 °C via a solution-mediated transformation pathway to produce form I. On drying, the optically transparent trihydrate laths transform to an opaque, micronized form I. The metastable trihydrate is characterized as a maximally solvated form that appears to defy Ostwald’s rule of stages and thus behaves fundamentally differently from the highly metastable polymorph form III.

Introduction

Acetaminophen (4-acetamidophenol; paracetamol) is a well-studied pharmaceutical compound that is known to exhibit polymorphism.1,2 Three crystal forms of the pure substance are known; one of those is a metastable bilayer structure, form III, that was characterized by a combination of high-throughput crystallization experiments and refinements of calculated structures from polymorph prediction.3,4 The relative thermodynamic stability of the polymorphs falls in the order of I > II >> III, and the interconversion of the forms follows the reverse order.5

Binary phases of acetaminophen have been made in which hydrogen bonding with amine base is exploited to generate chains of the 1:1 adducts, as well as a 2:1 acetaminophen/4,4′-bipyridine cocrystal.6 A hemisolvate with 1,4-dioxane has also been reported.7 Recently, crystalline monohydrate8a and trihydrate8b forms have been described. The procedure to prepare the trihydrate material involved quench-cooling an aqueous solution and capturing the resulting crystals in oil, enabling the single-crystal structures to be determined. Herein we report on the physical stability of the acetaminophen trihydrate and characterize form conversion in aqueous suspensions and on drying of isolated lath crystals.

Experimental Section

Reagents. Acetaminophen (lot 100K0197) was from Sigma-Aldrich. All reagents were used as received. Water was purified using a Fluid Solutions RODI system and tested to have 18.3 MΩ resistivity. Ice was prepared using a Scotsman icemaker and crushed to desired size.

Instrumentation. Powder X-ray diffraction experiments were carried out using a Rigaku Rapid-DMAX microdiffactometer. Raman spectra were recorded using a ThermoNicolet Almega Dispersive Raman spectrometer equipped with a 785 nm laser. Differential scanning calorimetric studies were run using a TA Instruments DSC Q1000. Optical microscopy was performed using a Zeiss Axiosplan polarized light microscope equipped with a digital camera.

Procedures. All crystallization experiments were carried out in 20 mL glass vials fitted with Teflon-sealed metal crimp caps. The vials were arrayed in aluminum blocks that were placed on temperature-controlled aluminum plates. Solutions of acetaminophen in water (20–40 mg/mL) were prepared by heating mixtures to 70 °C for about 30 min.8 Dissolution was monitored visually. After all of the solid acetaminophen had dissolved, the warm solutions (~10 mL) were filtered into 20 mL glass vials that had been incubated at 60 °C, through 0.2 µm PVDV syringe filters using 10 mL plastic disposable syringe bodied. The glass vials were immediately sealed and transferred to aluminum blocks that had been preheated to 60 °C. After all of the samples had been prepared and arrayed in aluminum blocks, the blocks were cooled to 0 °C over 12 h and then maintained at 0 °C until analyzed. In several samples, laths were observed and subsequently characterized as acetaminophen trihydrate. For seeding experiments, 30 µL of a suspension of acetaminophen trihydrate crystals, prepared by cooling a 30 mg/mL aqueous solution, was added to cooled solutions followed by gentle manual agitation.

The concentration of acetaminophen in solution in samples containing trihydrate and form I were measured by HPLC using a UV detector (λ = 244 nm). Samples used for HPLC analysis were prepared by carefully removing 500 µL of solution from above the crystals and diluting immediately to 250 mL with water in a volumetric flask. Samples were analyzed using a Thermo Hypersil-Keystone Betabasic C18 column on a Waters Alliance System HPLC and 82:15:3 (v/v/v) water/methanol/acetic acid as the mobile phase.

Results and Discussion

Crystallization of the Trihydrate by Cooling. Out of 40 crystallization attempts, five samples spontaneously produced the trihydrate. Other samples produced the monoclinic form I crystals from supersaturated solution within a week, but some samples did not spontaneously nucleate after more than one week. Samples that contained a nominal concentration of greater than 35 mg/mL acetaminophen generated form I acetaminophen, based on PXRD. Rapid cooling in a –20 °C freezer caused nucleation and growth of form I.
crystals. Furthermore, highly supersaturated solutions (40 mg/mL and above) generate only form I at 0 °C, but readily grow the trihydrate if seeded with trihydrate laths.

**Crystallization of the Trihydrate by Seeding of Saturated Aqueous Solutions.** Form I acetaminophen was dissolved in water by warming to 60–65 °C. Upon rapid cooling of the sample to 0 °C and subsequent seeding with <100 μL of a suspension of trihydrate (<3 mg assuming a homogeneous suspension of 30 mg/mL), the trihydrate quickly crystallized from the aqueous solution. Approximately 8 g of trihydrate were prepared in this manner. The formation of the trihydrate from seeded solutions was rapid: a thick suspension of trihydrate formed within 3 min. As little as 25 μL of suspension (or about 0.75 mg of trihydrate seed) sufficed to cause the entire vial to fill with trihydrate crystals. The crystals produced by seeding were identical by PXRD (Figure 1) and by Raman to the crystals produced by McGregor’s method. The suspension slowly settled to the bottom of the vial over 24 h, but the laths could be gently resuspended by swirling without incurring transformation to form I. As anticipated, suspensions at 0 °C containing form I crystals did not yield the trihydrate upon addition of seed crystal. However, even when seeded with form I crystals, suspensions in equilibrium with the trihydrate did not transform for several weeks in solution at 0 °C. When warmed above 5 °C, these solutions converted rapidly to large form I prisms. Accordingly, the trihydrate is stable in suspension below 5 °C. Solution generation of the trihydrate form requires a narrow window of temperature (ca. −2 to 5 °C). This observation may explain why the trihydrate has eluded researchers for over a century.

To determine if form II was an intermediate in the transformation of the trihydrate to form I, samples of the trihydrate were subjected to conditions thought likely to bias the transformation toward form II. Aliquots containing the trihydrate were quenched in toluene, or filtered under cold toluene after cooling the filtration funnel in ice. Toluene was chosen because of the apparent ability of this solvent to increase the likelihood of forming form II from evaporative and cooling recrystallizations. In no case was form II observed. Instead, mixtures of the trihydrate and form I were observed by PXRD without any indication of form II (Figure 1, panel I+T). This does not exclude the
possibility of form II existing as a fleeting intermediate, but it does bear upon the difficulty to be found in attempts to generate form II using the trihydrate as an intermediate.

**Crystallizations from Saturated Aqueous Solutions Containing Formulation Excipients.** It is well known that different crystalline forms of a drug can have dramatically different pharmaceutical properties, and hence there is significant regulatory scrutiny of physical form issues. Analysis of the potential formation of acetaminophen trihydrate in the presence of the excipients found in Children's Tylenol was studied. Aqueous solutions of acetaminophen (30 mg/mL) containing propylene glycol or glycerol were prepared. The two alcohols are inactive ingredients found in Children's Tylenol. The solutions were cooled and seeded with 100 μL of trihydrate suspension (nominal concentration of 30 mg/mL drug) to determine if these solutions would form the trihydrate. Trihydrate crystals were obtained from samples containing at least 50% water by volume. Pure water solutions were most stable toward trihydrate conversion to form I. As the amount of water decreased, the stability decreased and the material became highly susceptible to conversion into form I. Solutions containing less than 50% water by volume only produced form I. As expected, the water activity required to produce the trihydrate is significant. For reference, pediatric suspensions of Tylenol contain about 25% w/v glycerol, a propylene glycol component and about 160 mg/mL acetaminophen. Hence, the relevance of the trihydrate to commercial suspension formulations appears to be minimal.

**Crystallizations by Rapid Freezing.** Flash-frozen solutions of 30 mg/mL acetaminophen in water were generated by immersion of vials containing 10 mL of solution in liquid nitrogen. The frozen solutions were then allowed to warm slowly to 0–5 °C and then allowed to melt. Tiny lath crystals of about micron length, characteristic of the trihydrate, were observed. On further warming to room temperature, the microcrystals slurry converted to form I. When solid was scraped from the flash-frozen solution into a 30 mg/mL acetaminophen solution at 0 °C, the vial filled with small lath-like crystals which were identified by Raman as trihydrate. The laths prepared by scraping the frozen solution qualitatively appeared larger and more physically stable than those that came directly from the liquid nitrogen-quenched solution. In our hands, this method of making the trihydrate was more robust than slow cooling.

**Crystallizations by Seeding with Ice.** Inspection of the single-crystal structure of acetaminophen trihydrate does not indicate any ice-like arrangements of the waters of hydration within the structure. Since the trihydrate can crystallize by spontaneous nucleation at 0 °C and also by freezing saturated acetaminophen solutions (30 mg/mL) in water, the effect of seeding with ice was studied. Solutions of acetaminophen in water (30 mg/mL) were cooled from 60 to 0 °C over 12 h.
Several samples did not spontaneously crystallize and were maintained at 0 °C. One such sample was seeded with ice crystals obtained directly from an ice maker by adding two small ice pieces (both together fit on the tip of a 3 mm wide spatula). The resulting sample was swirled and returned to the aluminum block and maintained at 0 °C. No crystals were observed visually after 1 h at 0 °C, but form I crystals were observed after the sample was allowed to stand at 0 °C overnight. Two additional samples were seeded with ice that was precooled in liquid nitrogen for 10 min. In both experiments, two small pieces of supercooled ice were added to the solution, and the cold solution was swirled and returned to the aluminum block held at 0 °C. The phase of the ice was not determined. After about 5 min, laths of acetaminophen trihydrate filled the vial. The samples were confirmed to be the trihydrate by monitoring the conversion to form I in a drop of the suspension by optical microscopy. Crystallization of an additional sample was induced by adding liquid nitrogen directly to the saturated solution (no exogenous ice was used). After incubation of the sample at 0 °C for 15 min, crystals of form I were observed. These experiments show that acetaminophen trihydrate can nucleate in 30 mg/mL solutions by the addition of supercooled ice. However, they do not prove a templating mechanism, nor is such a mechanism suggested by the structure of the trihydrate.

Form Conversion in Suspension. Acetaminophen trihydrate crystals are only slightly more soluble than form I at 0 °C (49.5 µM for the trihydrate vs 49.0 µM for form I, at 0 °C). These data, obtained by HPLC of samples of supernatant in equilibrium, suggest that the trihydrate and form I are close to a transition point in stability around the freezing point of water. On warming of the sample to room temperature, form I crystals grow in the solution. Optical photomicrographs taken during slurry conversion are shown in Figure 2.

Form Conversion on Drying. When trihydrate laths were dried on filter paper at close to 0 °C, the normally clear, colorless crystals became opaque over the course of about 1 min. When manually manipulated, the resulting opaque crystals disintegrated into a fine powder that was identified as form I acetaminophen by PXRD and Raman spectroscopy. In a few cases, the conversion of the trihydrate to form I was observed and monitored by optical microscopy of rapidly filtered and transferred crystals (Figure 3). The transformation appears to take place along two crystallographically related planes in the single trihydrate crystal, although other examples indicate that this does not happen in all cases. The conversion resulted in polycrystalline pseudomorphic “laths” of form I. When touched gently with a probe the “laths” fell apart into many small particles ranging in size from 0.25 to 2 µm in diameter (along lath axis). This method of self-micronization of
form I acetaminophen may offer an alternative to milling methods.

On the basis of the published crystal structure and the data presented herein, the acetaminophen trihydrate structure appears to be a maximally solvated species with limited thermal stability. The form interconversion data of acetaminophen is summarized in Scheme 1. It is interesting to note that the trihydrate appears to go directly to form I, the thermodynamically stable polymorph. While it is not possible with the data presented to strictly rule out any existence of form II during the conversion of the trihydrate into form I, this behavior would seem to violate Ostwald’s rule of stages, which states that a metastable polymorph should form first. The trihydrate behaves fundamentally differently from the metastable form III polymorph, which transforms to form II (the metastable orthorhombic form). It is unclear as yet how the water leaves the structure on drying of the trihydrate. It is clear, however, that the drying front moves along two crystallographic planes. The porosity introduced by the expulsion of water from the structure yields a powder of form I, suggesting that the loss of water causes a density difference and this results in the collapse of the now unstable trihydrate structure to a more thermodynamically favorable anhydrous structure.

**Summary**

A study of the aqueous crystallization and transformation dynamics of acetaminophen trihydrate has been presented. The trihydrate form of acetaminophen can be reproducibly generated in the presence of commonly used alcohol excipients by seeding with either trihydrate or supercooled ice or alternately by flash freezing supersaturated aqueous solutions. We have shown how the trihydrate converts to form I through slurry conversion and solid-state (drying) mechanisms.

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**Supporting Information Available:** Raman spectra, powder X-ray diffraction patterns, differential scanning calorimetry traces, photographs, and optical micrographs are available free of charge on the Internet at http://pubs.acs.org.

**References**

8. Room temperature aqueous solubility of form 1 acetaminophen was measured to be 13.8 mg/mL at 20 °C. (b) Aqueous solubility was reported to be 12.8 mg/mL in Granberg, R. A.; Rasmussen, A. J. Chem. Eng. Data 1999, 44, 1391–1395.
9. Powder X-ray diffractograms of acetaminophen trihydrate were obtained from samples removed from the crystallization vials by pipeting directly into a glass X-ray capillary tube. The capillary was quickly mounted in the aluminum sample holder that had been cooled in liquid nitrogen. The sample holder was transferred to the diffractometer quickly and the pattern was recorded. It was important to keep the sample cold during the transfer, and to quickly obtain the powder pattern so as to avoid conversion into form I.
10. The experimental powder patterns were compared with one calculated from the single crystal structure using Materials Studio Version 2.1, 2001, Accelrys Inc.
12. Children’s Tylenol Suspension Liquid (made by McNeil Consumer) contains the active ingredient acetaminophen (160 mg/mL) and the following inactive ingredients: butylparaben, cellulose, citric acid, corn syrup, flavors, glycercin, propylene glycol, purified water, sodium benzoate, sorbitol, xanthan gum. In addition to the above ingredients cherry-flavored suspension contains D&C Red #40, bubble gum-flavored suspension contains D&C Red #3 and FD&C Blue #1.