Dehydration of Risedronate Hemi-Pentahydrate: Analytical and Physical Characterization

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ABSTRACT: Dehydration of hydrates of pharmaceutical active ingredients (pharmaceutical hydrates) may easily occur during storage or manufacturing. Loss of water may have little effect on the crystal lattice, produce less hydrated forms or possibly amorphous forms. Characterizing the effects of water loss on crystal hydrate forms is important for understanding the behavior of pharmaceutical hydrates throughout the manufacturing and storage processes. This study shows that exposure of the hemi-pentahydrate form of risedronate monosodium to gentle heating (60°C) or conditions of low relative humidity (<10% RH) results in the loss of 1 mole of channel-type water. Upon removal of the channel-type water, the crystal lattice adjusts producing a distinct phase characterized by X-ray, thermal, IR, Raman, and NMR data. Adjustment of the crystal lattice appears to compromise crystal integrity and can result in reduced crystallite and particle sizes.
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INTRODUCTION

Environmental variables encountered during the drug manufacturing process may effect the formation of different crystalline states of hydration for the active pharmaceutical ingredient of a drug product.[1] These different solid forms or hydrates can possess different physical properties such as differences in solubility, stability, bioavailability, dissolution rate, and particle habit. Water in pharmaceutical hydrates can be described by three different structural classes that include those residing in isolated lattice sites, lattice channel sites, or ion-coordinated sites.[2,3] In isolated lattice sites, water molecules are isolated from other water molecules due to contact with drug molecules. Water molecules forming lattice channel sites are in contact with other water molecules of adjoining unit cells along an axis of a unit cell. It has been shown that some channel water containing hydrates may undergo dehydration under conditions of low relative humidity (RH) or pick up water under conditions of high RH.[3] Ion-coordinated water participates in an ion-water bond which usually is much stronger than any hydrogen bonds present. In addition to the formation of different hydrates, a single hydrate form of an API may contain more than one structural class of water.[2]

Characterization of different hydrate forms of the bisphosphonate compound risedronate [1-hydroxy-2-(3-pyridinyl)ethylidene] bis [phosphonic acid] monosodium salt, which is prescribed for the treatment of osteoporosis, was recently reported.[4] Three different hydrate forms were characterized including a monohydrate, a hemi-pentahydrate, and a variable hydrate containing between 4 and 6 moles of water. The hemi-pentahydrate form, which is the commercial form, was found to contain two classes of water molecules including at least 1 mole of lattice-type (possibly ion-coordinated) and 1 mole of channel water. Channel water is characteristically mobile

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and may migrate into and out of the crystal lattice as a function of ambient humidity. This mobility was illustrated previously with vapor sorption data presented for the hemi-pentahydrate form of risedronate.[4] Over a RH range of 20–90%, the water content remains stable. As the RH is decreased from 9% to 5%, a weight loss of 5% occurs corresponding to the loss of 1 mole of water. The channel water then reenters the lattice as the humidity is increased from 13% to 20% resulting in complete rehydration.

In contrast to the removal of lattice water, the removal of channel water often leaves the crystal lattice relatively intact.[3] However, upon the removal of channel water from risedronate hemi-pentahydrate, thermal and spectroscopic data indicate the lattice undergoes an adjustment and the crystal integrity appears to be compromised. This study describes the formation of dehydrated risedronate by removing 1 mole of channel water with heat or desiccation and discusses the associated physical and chemical changes that occur. These changes are shown to be reversible, and the crystal lattice returns to its original state as the sample rehydrates.

EXPERIMENTAL

Materials

The hemi-pentahydrate was sourced from P&G Pharmaceuticals, Inc. commercial supply and used as received. The dehydrated material was prepared by desiccating hemi-pentahydrate over anhydrous calcium sulfate for at least 48 h. Rehydration was accomplished by exposing the dehydrated material to RH of >20% until rehydration was complete, typically within hours.

Analytical Methodology

Thermal Analysis

Simultaneous thermogravimetry and differential thermal analysis curves (TGA/DTA) were generated using a Seiko SSC/5200 custom equipped with a quartz glass window.[5,6] Samples (approximately 10 mg) were scanned under a dry nitrogen purge from 25 to 250°C at 5°C/min. Photomicrographs were obtained by mounting a microscope with a video feed above the quartz glass window. Acceptable depth of field and focus was achieved using top illumination, a 0.5 objective on the microscopy, and 10× magnification in the eyepieces and camera feed.

Infrared and Raman Spectroscopy

Fourier transform infrared spectra (FTIR) were obtained using a BioRad FTS-3000 spectrometer with 4/cm resolution. Sample desiccation was minimized by dispersing materials in both Fluorolube (4000–1350/cm) and Nujol (1350–400/cm) mulling agents. This sample preparation technique enables the collection of infrared spectra from the hemi-pentahydrate and dehydrated forms of risedronate in their native states. Fourier transform Raman spectra (FT-Raman) were obtained using a Nicolet FT Raman 960 spectrometer at 8/cm resolution. Localized heating due to absorption of the laser light is known to impact Raman spectra of the hemi-pentahydrate and dehydrated forms of risedronate. The laser intensity employed for spectral acquisition (0.5 W) and cumulative sample exposure (16 scans) were below the threshold known to cause spectral changes due to localized heating.[4] Samples were prepared by placing material to be analyzed into small quartz tubes and illuminating the sample with the laser only during spectral acquisition.

Solid-State NMR Spectroscopy

Cross-polarization/magic-angle spinning (CP/MAS) solid-state NMR (SSNMR) spectra were obtained using a Varian UnityINOVA 300 NMR spectrometer equipped with a Varian 7 mm CP/MAS probe.[7] Each sample was characterized by 121.4 MHz ^{31}P, 75.4 MHz ^{13}C, and 79.4 MHz ^{23}Na SSNMR spectroscopy. Chemical shifts were referenced externally for phosphorus to phosphoric acid, 85 weight % (neat) at 0.0 ppm; for carbon to hexamethylbenzene at 17.3 ppm;[7] and for sodium to 0.1 M NaCl (aq) at 0.0 ppm. The risedronate samples were not ground, and were packed into 7 mm silicon nitride rotors fitted with Torlon caps and spun at the rate of 5 kHz. Dehydration of hemi-pentahydrate was monitored first *in situ* by ^{31}P SSNMR spectroscopy using a rotor with a vented cap. Spectra were collected during probe heating from 20 to 60°C (10°C increments) and then during a 60°C hold, until no further changes were detected in the spectra. Dehydrated risedronate samples created by heating at 60°C in an oven or by desiccation were packed using a glove box under a dry nitrogen gas purge. CP/MAS ^{31}P and ^{13}C spectra were obtained for each sample. ^{13}C spectra were

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recorded with and without sideband suppression (TOSS[8]); also with and without proton dephasing.[9] For each material, the same Hartman-Hahn match was used, and the contact time was optimized for maximum signal intensity.[10] ^{23}Na spectra were collected using a Bloch decay with a 30° excitation pulse and a 10 s relaxation delay. ^{31}P sideband intensities were analyzed by the Herzfeld–Berger method[11] using the HBA program[12] to obtain chemical shift tensor information.

X-Ray Diffraction

X-ray powder diffraction was performed on the samples using the Bruker D5000 X-ray diffractometer. The D5000 was equipped with a 2.2 kW Cu anode X-ray tube, an Anton Parr TTK-1 low temperature stage, and high-speed position sensitive detector (PSD). Cu Kα radiation ($\lambda = 1.5418$ Å) was used to obtain all powder patterns. A dual foil, nickel filter was placed in the receiving path of the X-rays to remove the Kβ radiation. Risedronate sodium, hemi-pentahydrate material was mounted and analyzed on a front loading sample holder, without any special sample preparation. Environmental conditions for the analysis were manipulated to facilitate drying and rehydration of the sample without removing it from the instrument. The sample was dehydrated by heating the material to 60°C and holding for duration of the analysis. After dehydration, it was re-hydrated by allowing the material to cool and stabilize at room RH for 20 min. All scans were performed over the range of 3.5–40° 2 theta, at a 0.02° step size for 0.2 s/step.

Light Microscopy

Light micrographs were obtained using a Nikon Eclipse e600 Polarizing Light Microscope (PLM) with an Optronics 3-Chip color camera. Slides were prepared by mixing the powdered sample with low viscosity immersion oil, and the resulting dispersion placed between a clean glass slide and cover slip. Each prepared slide was examined using brightfield and slightly uncrossed polarized light using a Nikon PlanFluor 10×/0.30 objective.

Particle Size Analysis

Particle size of the hemi-pentahydrate and dehydrated forms of risedronate was determined using laser diffraction. Approximately 50 mg of each sample was introduced as a dry powder into Isopar V, a synthetic isoparaffinic oil. The resulting dispersions were analyzed using a Horiba LA-920.

RESULTS AND DISCUSSION

Thermal Analysis

Comparing fully hydrated risedronate to desiccated risedronate via thermal analysis clearly illustrates that desiccation results in loss of 1 mole of channel water from the molecule while leaving the remaining 1.5 moles of water intact. Representative thermal curves are provided below in Figure 1. The only difference in the TGA curves (Fig. 1A) is the initial mass loss of 5.2% observed from room temperature to approximately 70°C in the fully hydrated sample that is missing completely from the desiccated sample. This value is in agreement with theory for loss of 1 mole of water from the hemi-pentahydrate (5.16%). The higher temperature mass losses of the two samples match in both temperature and magnitude. A detailed interpretation of the TGA losses was provided in an earlier publication.[13,14] In the earlier study, the dehydration was assigned to loss of channel water based upon the temperature of the water loss (below the boiling point of water) and the observed variation in the loss profile as a function of scan rate and venting. In this study, the assignment was confirmed by the disappearance of this first dehydration step in the desiccated sample. Collectively, these data show how the 1 mole of channel water may be quantitatively drawn out of the crystal lattice either by gentle heating (as demonstrated by the TGA curve of the fully hydrated sample) or by desiccation at room temperature.

Unlike the TGA comparison, differences are observed in the DSC curves in addition to those anticipated for simple loss of the mole of channel-type water of hydration (Fig. 1B). The desiccated sample yielded a flat baseline through 90°C, over the same temperature range that a broad endotherm due to dehydration is observed for the fully hydrated sample, just as expected. At higher temperatures, additional changes are observed. The loss of the final mole of lattice-type water of hydration from the hemi-pentahydrate occurs just above 140°C.[13,14] In the desiccated sample, this dehydration occurs as a much sharper and larger endotherm than that observed for the fully hydrated sample. Subsequent endotherms due to

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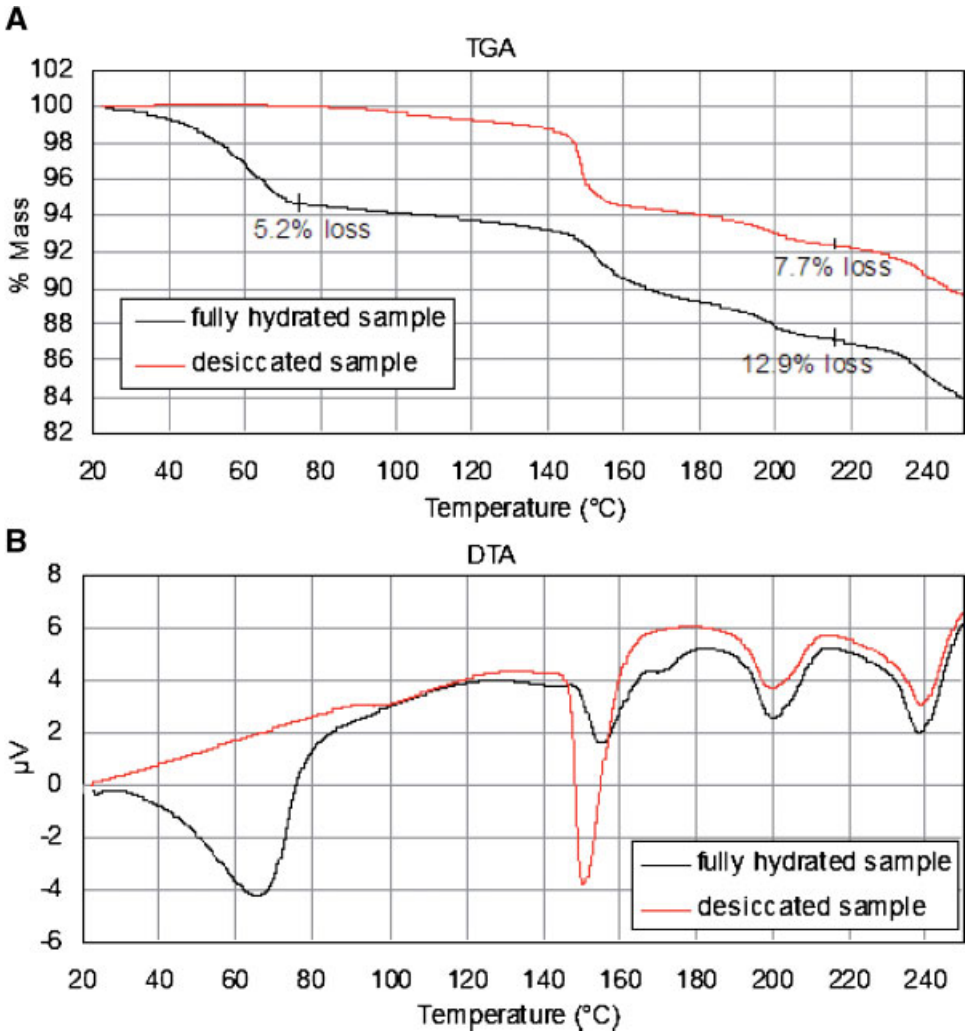

Figure 1. A: Comparison of TGA curves for untreated and desiccated samples of risedronate. Loss of 1 mole of channel water observed by 70°C in untreated sample but not desiccated sample. B: Comparison of DSC curves. Note lack of initial dehydration endotherm and difference in dehydration endotherm near 150°C for the desiccated sample.

dehydration of monohydrate formed *in situ* and degradation match between the untreated and desiccated samples.[13,14] The change in dehydration profile for loss of water from the isolated lattice site suggests that channel water loss upon desiccation results in a lattice adjustment in response to the missing water.

Use of a TGA/DTA equipped with a quartz glass window within the furnace wall and a microscope, enabled visualization of the impact the loss of channel water from the crystal. The series of photomicrographs provided in Figure 2 visually illustrate the macroscopic impact of drying upon the crystal and correspond to the first mass loss step observed in Figure 1A. Initially (25°C), the hemi-pentahydrate crystals are essentially clear with some noticeable internal fracturing. As the channel water is driven out of the crystal, it becomes more and more opaque, indicating some type of lattice adjustment to the loss of water and result fracturing. This is clearly beginning to occur with the initial onset of mass loss at 36°C. As the last of the channel water exits the crystal, noticeable expansion can be seen as the result of continued fracturing (56–75°C).

Infrared and Raman Spectroscopy

Infrared spectroscopy is particularly sensitive to the polar functional groups observed in

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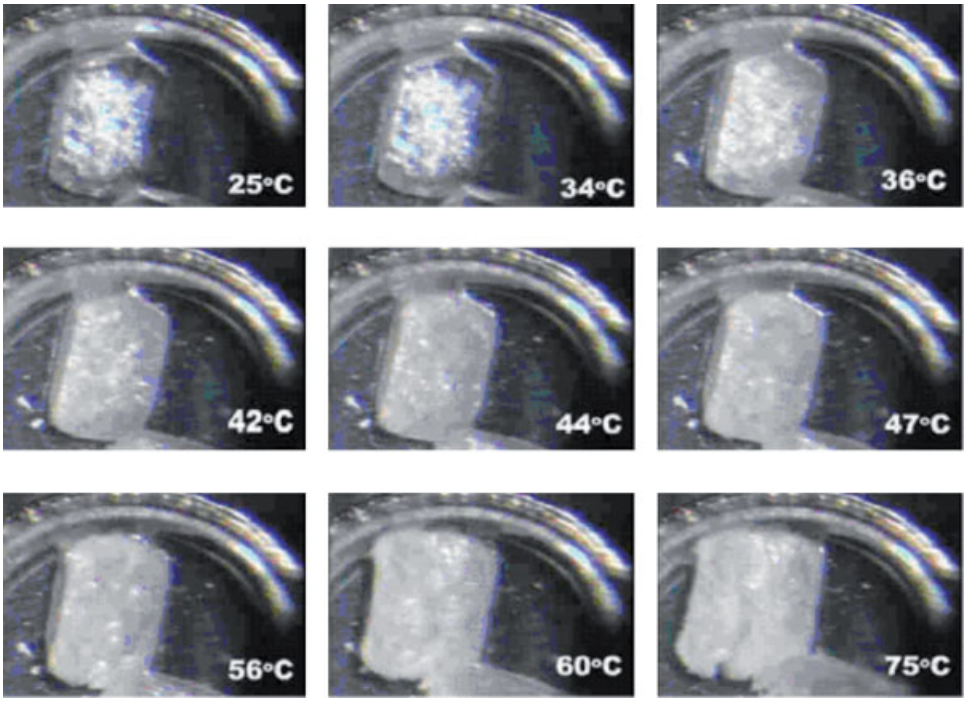

Figure 2. TGA/DTA/Microscopy photos of the impact of drying upon the hemi-pentahydrate. As the channel water driven off the crystal becomes more opaque (beginning at 36°C) and as the last of the channel water is driven off, the crystal expands in volume (56–75°C).

risedronate as well as water of hydration and its effect on hydrogen bonding. The infrared spectrum of the dehydrated form of risedronate compared to spectra from the hemi-pentahydrate and monohydrate forms of the material exhibit significant differences.[4] A comparison of infrared spectra from the hemi-pentahydrate and dehydrated forms of risedronate is illustrated in Figure 3A. There are significant changes in the O–H stretch region (~3000 to ~3600/cm) as well as throughout the spectra indicating significant differences in hydrogen bonding characteristics within the crystal lattice between the two different hydration states. The hemi-pentahydrate form exhibits two sharp, distinct, O–H stretch peaks at 3566/cm and 3618/cm. The shape and location of these peaks are indicative of constrained water in the crystal lattice in two separate environments. Dehydration of the hemi-pentahydrate form results in the disappearance of the peak at 3618/cm producing spectral evidence indicating water was removed from discrete locations in the crystal lattice. These spectral changes associated with dehydration of the hemi-pentahydrate form are easily observed by repeated spectral acquisition during sample dehydration (Fig. 3B) and are reversible upon rehydration of the material.

The O=P–OH group produces broad IR bands in the spectral region between 2000 and 2725/cm that are complex with multiple overlapping peaks due to multiple O=P–OH groups in the molecule. Changes observed upon dehydration of hemi-pentahydrate show shifting and changes in relative peak intensities in this spectral region. The largest O=P–OH peak at 2105/cm is more intense in the hemi-pentahydrate spectrum than the dehydrated form possibly due to delocalization of hydrogen bonding associated with the hydration state of the material. The fingerprint region of the spectrum between 900 and 1215/cm is dominated by peaks characteristic of the phosphonic acid group overlapped with pyridine ring deformation bands. Dehydration of the hemi-pentahydrate form produces a very large decrease in intensity at approximately 1150/cm associated with the asymmetric P=O stretch. The symmetric P=O stretch observed as an intense shoulder band at approximately 1103/cm in the hemi-pentahydrate broadens and shifts in frequency due to dehydration. Differences observed in spectral features associated with the O=P–OH and P=O groups

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