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Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 1080–1088
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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A study of variable hydration states in topotecan hydrochloride

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Received 2 July 2005; received in revised form 23 August 2005; accepted 31 August 2005
Available online 20 October 2005

Abstract

Topotecan hydrochloride, a pharmaceutical compound developed as a treatment for cancer, exhibits variable hydration states in a crystalline solid form chosen for manufacturing. This variability requires additional controls for successful development, and presents a characterization and detection challenge for analytical methods. In this study, overall water content was determined by Karl Fischer titration and thermogravimetric analysis (TGA) on topotecan HCl equilibrated at different relative humidity levels. These results, when combined with information obtained from dynamic water vapor sorption and differential scanning calorimetry (DSC), indicate that this form of topotecan HCl contains 3 mol of water integral to the crystalline structure and up to two additional moles of water depending on the relative humidity. Powder X-ray diffraction experiments did not detect significant differences in topotecan HCl samples equilibrated at trihydrate and pentahydrate states, and showed that the crystal lattice dimensions are not affected unless the form is dried below the trihydrate state. This behavior is typical of crystal structures with channels that can accommodate additional loosely bound water. To study the role of the loosely bound water in the crystal structure in more detail, solid-state ^{13}C and ^{15}N nuclear magnetic resonance (NMR) were used to examine the differences between the hydration states. Both the trihydrate and pentahydrate states yielded similar solid-state NMR spectra, consistent with the lack of change in the crystal lattice. However, minor but readily detectable differences in the ^{13}C spectra are observed with changes in water content. Interpretation of this data suggests that the loosely bound channel water is hydrogen-bonding to specific portions of the topotecan parent molecule. Topotecan HCl trihydrate was hydrated with D_2O vapor to confirm the nature and location of the channel water using ^{13}C and ^{2}H solid-state NMR. Despite the detectable association of the channel water with hydrogen bonding sites on the topotecan molecule, ^{2}H quadrupolar echo experiments indicate that the channel water is highly mobile at room temperature and at $-60\,^{\circ}C$.

Keywords: Non-stoichiometric channel hydrate; Vapor sorption; Solid-state NMR; Deuterium exchange; Thermal analysis; Powder X-ray diffraction

1. Introduction

The study, control, and analysis of polymorphism have become an integral part of process development for pharmaceutical manufacturing. Polymorphism is the ability of a solid compound to exist in more than one crystalline form with the same covalent chemical structure, but different supra-molecular structures and ordered arrangements of molecules within the crystalline lattice. Polymorphs of the same compound often have different physical properties, such as melting point and solubility [1,2]. In addition to exhibiting polymorphism, many drug molecules form hydrates and organic solvates, which themselves can show polymorphism. Although salt formation can sometimes simplify the situation, many molecules do not form stable or pharmaceutically acceptable salts. In some of these cases, hydrates are the most reasonable choice for further development, as evidenced by the large number of late-stage and marketed pharmaceutical hydrates [3,4]. Certain hydrates can be classified as so-called "channel" hydrates if they can reversibly dehydrate and hydrate with changes in temperature and humidity while retaining the same basic crystalline structure (channel hydrates are also referred to as variable hydrates, non-stoichiometric

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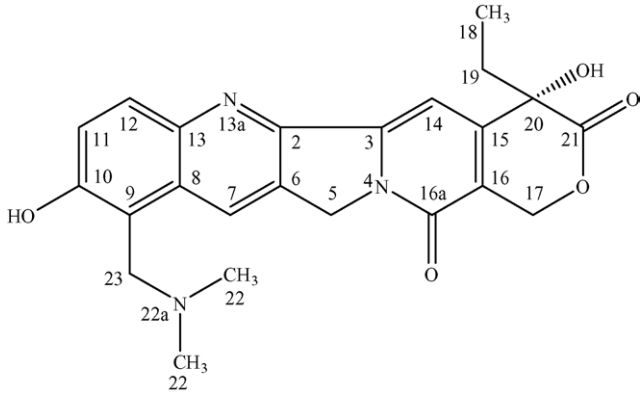
doi:10.1016/j.jpba.2005.08.032

hydrates, and isomorphic desolvates). Channel hydrate behavior is so named because it is generally caused by the presence of 5–10 Å diameter channels in the crystalline lattice. The drug molecule serves as a host, and the channels accommodate guest molecules like water (or other solvents) that enter and interact via hydrogen bonding or other forces [4]. The water molecules typically form extended networks within the lattice channels, or coordinate with metal ions in ion-associated hydrates [5–7]. Some channel hydrates can expand their overall cell dimensions to include additional water molecules, and are known as expanded lattice channel hydrates in the nomenclature of Morris and Rodriguez-Hornedo [6]. Others retain their unit cell dimensions even after uptake of several molar equivalents of water. When encountered, channel hydrates can be a difficult prospect for development because of the additional effort needed to ensure that they contain a predictable molecular composition and can be dispensed quantitatively. Channel hydrates can also cause concerns with respect to critical parameters like stability and solubility. However, channel hydrates can otherwise be thermodynamically favored, stable forms, and can have many other desirable properties for drug development. A number of channel hydrates have been reported and studied, including forms of cephalexin monohydrate [8], ampicillin trihydrate [9], cromolyn sodium (disodium cromoglycate) [10], caffeine [11], theophylline [11], arzoxifene [12], and sildenafil citrate [13].

The existence of a channel hydrate, when suspected, is often initially confirmed by vapor sorption studies, possibly including both dynamic and static methods with water vapor and other solvents. Vapor sorption experiments are usually attempted after reviewing the data obtained by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), which can be the first indicators of a variable hydration state. Thermal and vapor sorption methods are used in conjunction with powder X-ray diffractometry (PXRD), vibrational spectroscopy, and solid-state nuclear magnetic resonance (NMR). Changes in X-ray diffraction patterns can determine if the lattice expands or contracts with changing relative humidity and temperature, and if the hydration and dehydration processes observed by gravimetric vapor sorption isotherms are reversible. Another technique that is especially useful in channel hydrate analysis is solid-state NMR, a non-destructive technique that yields spectra with an NMR resonance for each magnetically non-equivalent carbon site, which for a crystalline material depends on the number of NMR-active nuclei per molecule and the number of molecules in the crystallographic asymmetric unit [14,15]. The chemical shift reports on the electronic environment of the NMR nucleus. The sensitivity and high information content of NMR offers a number of possibilities for the study of channel hydrates. Hydration effects can sometimes be directly observed in ^{13}C and ^{15}N solid-state NMR spectra, as in a recent study that detected hydrogen-bond formation to a citrate anion upon increasing the hydration state, along with conformational effects on a propyl sidechain on the parent molecule [13,16]. The relaxation times of NMR-active nuclei, including ^{1}H and ^{13}C, can also be used to probe motional changes in channel hydrates. For example, studies of thiamine hydrocloride monohydrate found that the loss of water led to a 5% reduction of the unit cell volume and to a slight increase in the free volume in the lattice [17]. In this case, ^{1}H and ^{13}C solid-state NMR T_1 relaxation measurements were able to detect site-specific increases in molecular motion that were directly related to water hydrogen bonding sites.

The present work involves the study of a variable hydrate of topotecan hydrochloride. Topotecan (also known as 9-(*N*,*N*-dimethylaminomethyl)-10-hydroxycamptothecin) inhibits DNA topoisomerase I, an enzyme that alters DNA topology in eukaryotic cells and affects DNA replication, transcription, and recombination. Topotecan shows efficacy against several solid tumor cancers, particularly ovarian cancer, esophageal cancer, and small-cell and non-small cell lung carcinoma in humans [18]. The chemical structure of the free base of topotecan is given below, along with a numbering scheme prevalent in the literature:



Topotecan is marketed as Hycamtin^{TM} and is currently administered via intravenous infusion. During the development of a solid oral dosage form, a detailed evaluation of novel topotecan crystalline forms was performed. Under a wide range of temperature, co-solvent, and water activity conditions, a crystalline form designated Form II was found to be particularly desirable. It was identified as a channel hydrate soon after its discovery.

Aspects of the development and characterization of topotecan HCl Form II relating to variable hydration are discussed here, including thermal and vapor sorption analyses, powder X-ray diffractometry (PXRD) and ^{13}C solid-state NMR. The characterization of Form II of topotecan hydrochloride was necessarily accomplished without the aid of single-crystal X-ray diffraction, because a single crystal of suitable size and quality could not be grown. In addition, a new NMR approach to the study of pharmaceutical channel hydrates is also explored, involving the use of ^{2}H-labeled water as an NMR tracer for further study of the water environment in the lattice. Deuterated water has been used to study the dynamics of channel water in hydrates via Raman spectroscopy [11]. Although not generally employed in pharmaceutical analysis, ^{2}H NMR has been widely used to study water dynamics and kinetics in solid hydrates, and is a proven method for studying these effects (along with ^{17}O NMR) [19,20].

2. Experimental

2.1. Materials

Topotecan free base is synthetically produced from 10-hydroxycamptothecin via a Mannich reaction [21]. The final particle-forming step of the process is the precipitation of the monohydrochloride salt from a methylene chloride and methanol solution of the free base by addition of concentrated aqueous HCl in 1-propanol. Topotecan HCl Form II can be prepared by dissolving the HCl salt reaction mixture in a solvent system of 2:1 (v/v) acetone/0.05N aqueous HCl at 58 °C. This solution is cooled at an initial rate of 1.0 °C/min down to 40 °C, at which point the solution is seeded with 0.1% (w/w) of the Form II polymorph (based on crude product mass). Following a hold period which allows the seeds to fully grow, the solution is cooled at 0.25 °C/min until it reaches 0 °C. Cooling at this slow rate after the seed growth period helps ensure that nucleation of a different polymorph cannot occur. The reaction product, topotecan monohydrochloride Form II pentahydrate, is isolated by filtration and dried at 32 °C and 150–200 mmHg absolute pressure for ∼40 h while passing a stream of nitrogen through the vessel. At the conclusion of this process, the topotecan HCl is dried to Form II and isolated as a yellow solid. It should be noted that the use of seeding is not required to obtain the Form II polymorph in this process. For the present hydration study, synthetically prepared topotecan HCl was milled and subsequently hydrated to various levels as discussed in the following sections. The milling process used here was not found to introduce detectable amorphous content as determined by water vapor sorption experiments and PXRD.

2.2. Vapor sorption and hydration experiments

Dynamic water vapor sorption experiments on topotecan HCl Form II were performed on a dynamic gravimetric vapor sorption (DVS) instrument from Surface Measurement Systems Ltd. at 25 °C. Each humidity point tested was held for a 2-h period. Static water vapor sorption samples were prepared by storing samples in sealed glass containers with saturated salt solutions (to control humidity), pure water, or dessicant. Samples were stored for a minimum of 87.5 h and a maximum of 114 h. The composition of the solutions in the sealed dessicators was as follows: 100% RH (pure H_2O); 84.3% RH (saturated KCl); 73.8% RH (saturated $NaNO_3$); 57.7% RH (saturated NaBr); 52.9% RH (saturated $Mg(NO_3)_2$); 42.8% RH (saturated K_2CO_3); 33% RH (saturated $MgCl_2$); 10.2% RH (saturated LiCl); and ca. 0% RH (anhydrous calcium sulfate desiccant) [22]. A hydration experiment with D_2O at 73.8% RH was performed in the same manner as the other experiments, starting from a sample initially stored at 33% RH.

2.3. Thermal analysis and water content

Karl Fischer titrations were carried out on a Metrohm 756-KF coulometer at ambient temperature and humidity. DSC experiments were carried out on a TA Instruments Q1000 system. A N_2 flow rate of 50 mL/min was used. Sample sizes ranged from 0.5 to 3.0 mg, and the heating rate was 10 °C/min. TG analysis was also performed on the samples using a TA Instruments Q500 system, again using a 10 °C/min heating rate. The TGA sample sizes were in the range of 5–20 mg.

2.4. Powder X-ray diffraction

PXRD patterns were measured using a Phillips X'Pert Pro diffractometer. Samples were gently flattened onto a zero-background silicon holder. A continuous 2θ scan range of 2°–35° was used with a Cu Kα radiation source and a generator power of 40 kV and 40 mA. A step size of 0.0167° per 2θ step with a step time of 10.16 s was used. Samples were rotated at 25 rpm and all experiments were performed at room temperature.

2.5. Infrared spectroscopy

For vibrational analysis, a Nicolet Magna 760 Fourier transform infrared (IR) spectrometer, equipped with a dTGS (deuterated triglycine sulfate) detector, was used to obtain IR spectra at a resolution of 4 cm^{-1}. At least 100 scans were collected for each spectrum. Samples were prepared by diluting approximately 2 mg of topotecan hydrochloride into 300 mg of dried potassium bromide (KBr), and pressing for three minutes into pellets. IR spectra were recorded at room temperature.

2.6. NMR spectroscopy

A Bruker AMX2-360 spectrometer was used for solid-state NMR at ^{15}N, ^{13}C and ^{1}H frequencies of 36.496, 90.556 and 360.097 MHz, respectively. A 7-mm double-resonance MAS (magic-angle spinning) probe was employed for ^{13}C and ^{15}N experiments. Approximately 150 mg of each sample was packed into 7-mm outer diameter magic-angle spinning rotors, sealed with a plug and a drive tip, and spun at 5 kHz ± 2 Hz under active control. Cross-polarization from ^{1}H to ^{13}C utilized a 2-ms ^{13}C RF power ramp from 30 to 40 kHz to enhance signal-to-noise and improve reproducibility [23]. Spinning sidebands were suppressed using a five-pulse TOSS (total suppression of sidebands) pulse sequence [24]. ^{1}H decoupling was performed at ∼60 kHz using the TPPM pulse sequence with an optimized pulse width of 10 μs [25]. One rotor period of non-quaternary suppression (NQS) editing was used to remove signals from rigid methine and methylene carbons [26]. Spectra were referenced to TMS using hexamethylbenzene as a secondary ^{13}C standard [27]. The ^{13}C spectra shown here were the result of 3888 averaged scans and used a 10 s relaxation delay. The ^{15}N experiments used the basic CP-MAS pulse sequence spinning at 5 kHz and with a contact time of 3 ms, without sideband suppression. Spectra were referenced to nitromethane via an external ammonium chloride secondary standard [28]. Each of the ^{15}N spectra was the result of 16,384 scans with a 5 s relaxation delay. The sample temperature was maintained at 298 K for the ^{13}C and ^{15}N experiments. An exponential window function of 10 Hz was applied to ^{13}C and ^{15}N data prior to Fourier transformation. Static quadrupolar echo spectra [29] were acquired on the same 7-mm MAS probe

at 55.282 MHz with the rotor stopped and the MAS drive gas disconnected. A deuterium pulse width of 3 μs, an echo delay of 30 μs, and a relaxation delay of 1 s were used, and 1024 scans were collected. Variable temperature (VT) control was achieved using a Bruker temperature controller and liquid nitrogen heat exchanger. An exponential window function of 500 Hz was applied to the data after a suitable left-shift to compensate for the echo delay.

Chemical purity and spectral assignments for topotecan HCl were determined by ^{1}H and ^{13}C solution-state NMR. A Bruker Avance spectrometer operating at 400.13 MHz for ^{1}H NMR was used for this task. Solution-state ^{1}H and ^{13}C assignments were determined at 298 K in d6-DMSO solution using standard 1D and 2D NMR experiments including ^{13}C spectral editing and short and long-range heteronuclear correlation experiments [30].

3. Results and discussion

3.1. Form stability experiments

Form II was studied by Ostwald ripening from mixtures of organic solvents with water or 0.1N HCl. It was found that Form II was almost always obtained from organic solvent/water ratios of 6 to 1. Although topotecan HCl is generally insoluble, slow evaporation experiments were possible in water, dimethylsulfoxide and methanol. However, these experiments produced amorphous topotecan HCl. During the course of the form screen and stability experiments, PXRD and IR were used as the definitive form tests. The results of the screening suggested that Form II was a stable and developable polymorph of topotecan HCl.

3.2. Dynamic and static water vapor sorption experiments

The hydration of Form II was also investigated carefully to ensure it was both a suitable form for manufacture and a stable hydrate under a wide range of water content and solvent composition conditions. Fig. 1 displays the dynamic vapor sorption data collected on this form. The first adsorption–desorption cycle indicates that the hydration state approaches a pentahydrate at 90% RH. The sorption of approximately 2 mol of water from 40 to 90% RH appears reversible, providing evidence that this form is a channel hydrate. At least three of the waters appear to have strong lattice bindings, indicated by the rate change and rapid loss of mass below 15% RH. The second and third desorptions ran to 0% RH, and the irreversible re-hydration behavior of the third adsorption cycle suggested that a partial polymorphic form change had occurred (this was confirmed by PXRD analysis). Based on the distinct steps observed in the third rehydration isotherm, an amorphous to crystalline conversion may also be occurring in the intermediate humidity region. However, the resulting form, which co-incidentally nears a pentahydrate state at 90% RH, was actually a mixture of undesired crystalline forms and was no longer pure Form II, even after storage at higher RH for 1 month.

In Fig. 2, the results of static vapor sorption studies are shown. The water content determined by TGA and KF is plotted for each sample. The results are consistent with the dynamic water

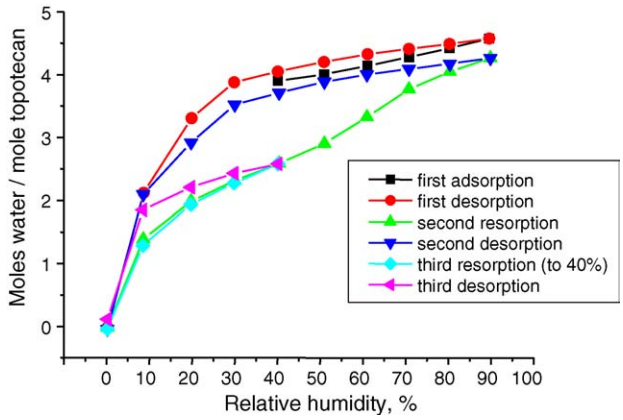


Fig. 1. Dynamic water vapor sorption data for topotecan hydrochloride Form II at 25 °C. The hydration state approaches a pentahydrate at 90% RH. Three moles of water appear to have lattice bindings, based on their rapid loss below 20% RH. Water adsorption from 40 to 90% RH appears reversible, indicating that these 1.5–2 mol are channel water. Water adsorption below 40% RH is not reversible, probably because of a gradual collapse of the lattice and formation of an amorphous phase.

vapor sorption data, given inaccuracies inherent in the analysis methods and kinetic effects. Because of the similarities in the water content obtained in the static and dynamic experiments, it can be concluded that the static vapor sorption experiments achieved equilibrium, given that the static samples were exposed for a period approximately fifty times longer than that used in the dynamic experiments. It should also be noted that the sample stored at 0% RH did not achieve the same anhydrous level seen in the dynamic experiment, but instead had 1–2 mol of water present. There are two likely reasons for this discrepancy. First, the calcium sulfate desiccant used to achieve 0% RH probably only reached 1–2% RH. Second, and more importantly, the samples stored at low RH were known to contain amorphous content (see below), and most likely adsorbed a significant amount of water before the KF and TGA experiments could be initiated. Two representative samples were retained for subsequent thermal, diffractometric and spectroscopic analysis: an approximate

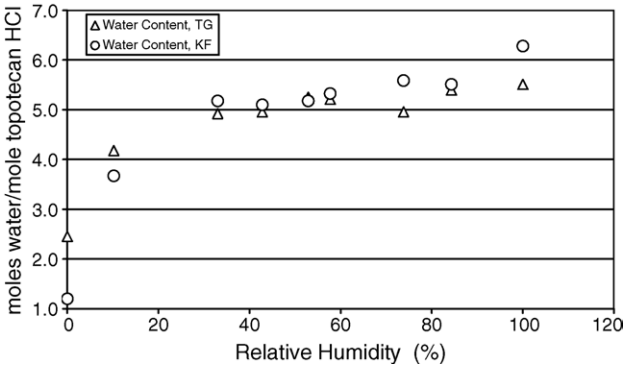


Fig. 2. Water content of topotecan hydrochloride Form II samples after 1 week of exposure in a humidity chamber at 25 °C, as assessed by both TGA (triangles) and KF titration (circles). The water content reaches a maximum of 5.0–5.5 mol of water after storage between 30 and 85% relative humidity. Unlike the dynamic water vapor sorption data, an anhydrous level was not achieved in these experiments (see text).

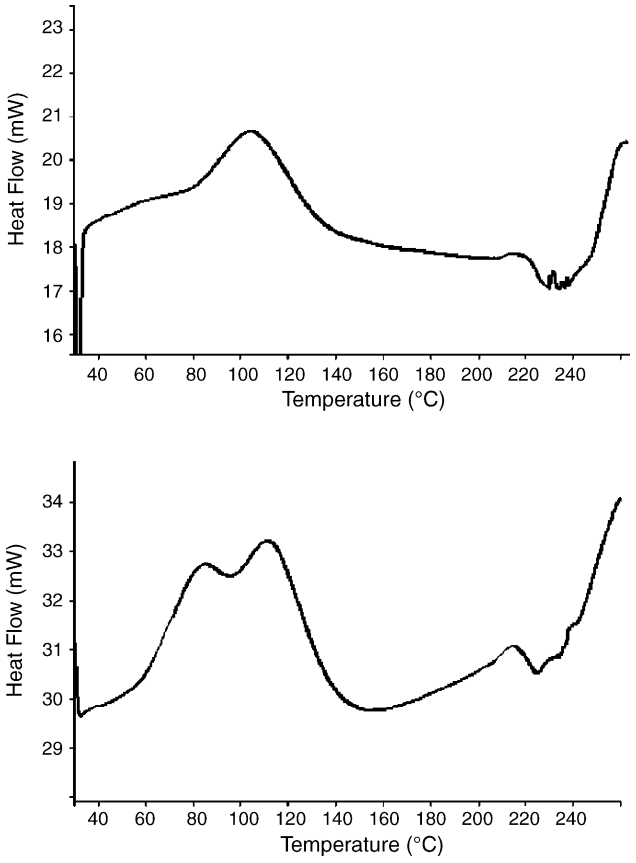


Fig. 3. DSC traces for topotecan hydrochloride Form II equilibrated for 144 h to the trihydrate state (top) and the pentahydrate state (bottom). Endothermic heat flows are plotted upwards. The peak temperature of the first endotherm in the pentahydrate DSC trace (∼85 °C) and its disappearance in the sample equilibrated to a trihydrate state suggests that this event corresponds to the loss of loosely bound channel water. The higher temperature event (peak at ∼108 °C) corresponds to the loss of the more tightly bound lattice water. Melting onsets at ∼205 °C.

trihydrate state with a water content of 11.0% (w/w), and a pentahydrate state with a water content of 15.9% (w/w).

3.3. Thermal analysis

The TGA of topotecan HCl samples showed the expected water weight loss by 150 °C (depending on the hydration state) prior to the onset of rapid decomposition at temperatures greater than 230 °C. The channel water and lattice water could not be distinguished by TGA. However, the DSC results in Fig. 3 are helpful in confirming the nature of water molecules in the lattice structure. The DSC trace from the trihydrate Form II sample showed a desolvation endotherm beginning at 46.6 °C and reaching a peak at 105.3 °C (ΔH = 269.5 J/g), followed by a melt with an onset of 206.6 °C and a peak of 217.6 °C (ΔH = 18.4 J/g). The pentahydrate Form II sample also showed a composite endotherm for desolvation starting at 56.2 °C and reaching a peak at 111.6 °C (ΔH = 364.0 J/g), with a melt onset of 204.8 °C and a peak of 214.6 °C (ΔH = 12.0 J/g). The endotherm with an onset below 100 °C appears to have a composite nature in both cases, but this effect is greatly exacerbated for the higher water content material. The composite pentahydrate thermal event in

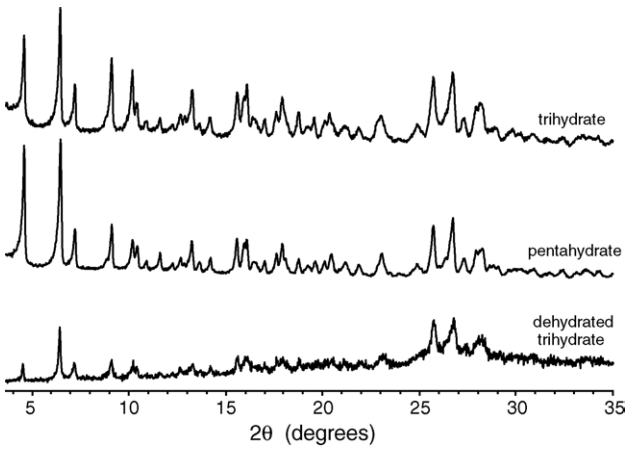


Fig. 4. PXRD patterns of topotecan hydrochloride Form II equilibrated for 144 h at the trihydrate state (top) and the pentahydrate state (middle). Patterns were measured at 25 °C. No significant changes are observed between the trihydrate and pentahydrate states, indicating that the crystal structure is static; i.e. there is no evident expansion or change of the crystalline lattice to accommodate the extra water. Drying below the trihydrate stage leads to a similar pattern, but with a substantial reduction in diffraction intensity and loss of peak resolution, indicating a loss of crystallinity (bottom). The pentahydrate pattern was slightly more intense and has been scaled by a factor of 0.8 relative to the trihydrate pattern. The dehydrated trihydrate pattern was weak and has been scaled up by a factor of 5.

Fig. 3 consists of two distinct endotherms, which are interpreted as representing hydration water with different binding energies. The first endotherm nearly disappears upon drying to trihydrate, without affecting product crystallinity. This behavior, in addition to the studies described above, provides further evidence that two types of water are present in the crystal lattice. The first type of water evolves at a temperature below 100 °C, suggesting it is loosely bound channel water. The second type of water exhibits a maximum evolution above 100 °C, indicating that it is more tightly held than the first water type. The quantitative method of Grant and co-workers can be used to estimate the water stoichiometry directly from the enthalpy changes observed in desolvation [9]. Using the specific enthalpy of vaporization of water as 2261 J/g, the trihydrate and pentahydrate are found to have 3.2 and 4.5 mol of water, respectively, in reasonable agreement with the KF and TGA results. Most importantly, the ability to clearly detect water in the lattice makes DSC a useful test for analysis of hydration in topotecan HCl Form II samples.

3.4. Powder X-ray diffraction

The PXRD patterns for Form II samples in the trihydrate and pentahydrate states are compared in Fig. 4. The patterns are virtually indistinguishable except for some intensity differences that could be caused by slight changes in crystallinity, packing or preferred orientation. It was concluded that the trihydrate and pentahydrate states are isomorphic, and that the unit cell does not undergo any detectable change in size to accommodate the extra 2 mol of water. Topotecan HCl Form II is classified as a lattice channel hydrate of the non-expanded variety [6]. The diffraction pattern of a dehydrated trihydrate sample, stored with desiccant

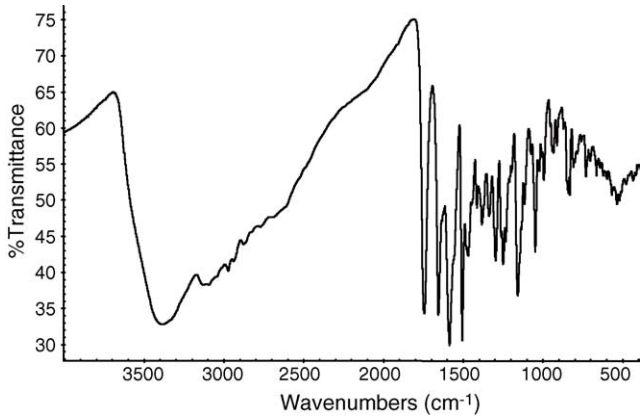


Fig. 5. FTIR spectrum of topotecan HCl Form II pentahydrate. Water of hydration is observed in the 2500–3700 cm^{-1} region of the spectrum, but was not found to be a distinctive measure of hydration state.

for 1 day, is also shown in Fig. 4. This sample was analyzed by KF and TGA and found to have a stoichiometry corresponding approximately to a dihydrate. A significant broadening of the peaks, as well as a five-fold loss of intensity, shows that the Form II lattice collapses to a semi-crystalline or amorphous under-hydrated state (although the pattern of Form II is still recognizable). This collapse was not found to be reversible by PXRD upon re-hydration for 1 month at higher humidity (73.8% RH); instead, as noted above, a mixture of undesired polymorphic forms was detected.

3.5. IR spectroscopy

IR spectroscopy of topotecan HCl Form II was used to confirm its form and distinguish it from other undesired polymorphs and solvates. However, IR spectra were not as useful for characterizing the level of water content. A non-distinct, broad variance in the region from 2500 to 3700 cm^{-1} was generally observed but was not a reliable indicator of hydration state. No detectable effects on the sharp vibrational bands of the parent molecule occurred with changes in water content. The IR spectrum of the pentahydrate form is shown in Fig. 5. Because of the broad changes in the higher frequency area of the IR spectrum caused by variable water content, the second derivative of the IR region from 1500 to 1800 cm^{-1} was chosen as a definitive test for the characterization of the crystalline form. The characteristic second-derivative frequencies of Form II in this spectral region are given in Table 1.

3.6. NMR spectroscopy

The ^{13}C solid-state NMR spectra of topotecan HCl trihydrate and pentahydrate are shown in Fig. 6. The spectra show that the crystal structure is complex, with at least two (and probably more) molecules in the crystallographic asymmetric unit. By comparison with the solution-state ^{13}C data given in Table 2, assignments can be made, although overlap limits some assignments to ~5 ppm wide regions of the spectra. Minor but distinctive changes are observed in the ^{13}C spectra upon conversion of the trihydrate to the pentahydrate state. These are denoted by arrows in Fig. 6. The spectrum of a second pentahydrate sample, prepared specifically for the NMR analysis by exposure of the trihydrate to D_2O vapor, is also shown in Fig. 6. In general, the ^{13}C spectrum of the D_2O pentahydrate resembles that of the protic pentahydrate, with the exception of a clear shift for the new peak in the ester region of the ^{13}C spectrum (C21). This peak shifts from 172.9 ppm in the trihydrate to 173.4 ppm in the pentahydrate, and then to 174.0 ppm in the D_2O pentahydrate. This behavior is consistent with formation of a hydrogen bond to a C21 carbonyl oxygen acceptor. In addition, both pentahydrate spectra show shoulder peaks for carbon positions C21, C18, C16, and C13. Interestingly, the first three of these sites are situated near the lactone ring of topotecan, and C13 is located next to the pyridyl nitrogen, a hydrogen-bond acceptor. All of these sites are likely locations for water molecules in a crystal lattice, and the results suggest that labile water molecules (when present) can interact with these sites enough to alter their NMR chemical shielding environment. The dipolar-dephased (NQS-edited) ^{13}C spectra were also obtained and are shown in Fig. 7. A reduction in dipolar dephasing is seen upon D_2O incorporation for some of

Table 1
Infrared band assignments for the second derivative of the characteristic spectral region (1500–1800 cm^{-1}) of topotecan hydrochloride Form II

IR frequency (cm^{-1})	Assignment
1754	C=O lactone
1745	C=O lactone
1740	C=O lactone
1658	C=O amide I
1649	C=N aromatic
1596	C=C aromatic
1584	C=C aromatic
1506	C=C aromatic

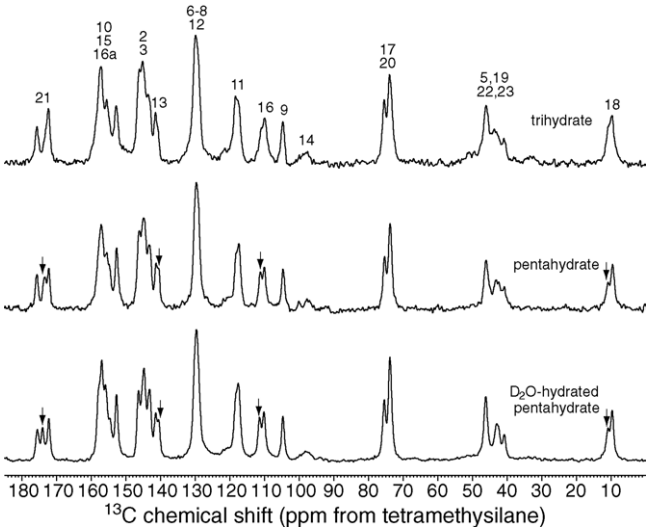


Fig. 6. Comparison of the ^{13}C solid-state CP-TOSS NMR spectra of topotecan HCl Form II trihydrate, pentahydrate, and deuterio-pentahydrate equilibrated for 144 h. Spectra were obtained at 25 °C. Assignments are numbered in reference to the structure of topotecan given in the text. Notable differences between the spectra are indicated with arrows.

Table 2
^{13}C NMR assignments for topotecan HCl in d6-DMSO solution

Position	^{13}C chemical shift
18	7.71
19	30.27
22	42.37
23, 5	50.05, 50.34
17	65.17
20	72.33
14	96.18
9	108.32
16	118.37
11	122.14
7	126.73
8	128.78
6	130.06
12	132.74
13	143.32
3	145.43
2	149.29
16a	150.00
15	156.74
10	156.99
21	172.40

Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS).

the same resonances that also showed changes in the ^{13}C spectra. The overlapped C17/C20 and C10/C15/C16a sites show the most distinct changes in dipolar dephasing behavior upon deuteration, in the form of notable signal increases relative to the rest of the spectrum (marked with arrows in Fig. 7). This suggests that vapor-exchanged deuterium oxide molecules also have access

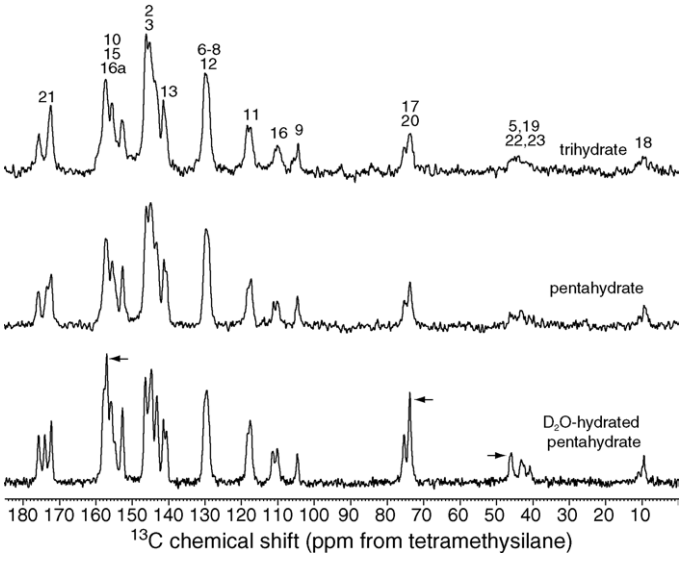


Fig. 7. Comparison of the ^{13}C solid-state CP-TOSS NMR spectra of topotecan HCl Form II trihydrate, pentahydrate, and deuterio-pentahydrate at 25 °C, with one rotor period (200 μs) of dipolar dephasing for non-quaternary carbon suppression (NQS). Assignments are numbered in reference to the structure of topotecan given in the text. In the bottom spectrum, a decrease in dipolar dephasing is seen upon D_2O incorporation for some resonances (denoted by arrows), which may have their dephasing behavior altered by proximity to mobile D_2O.

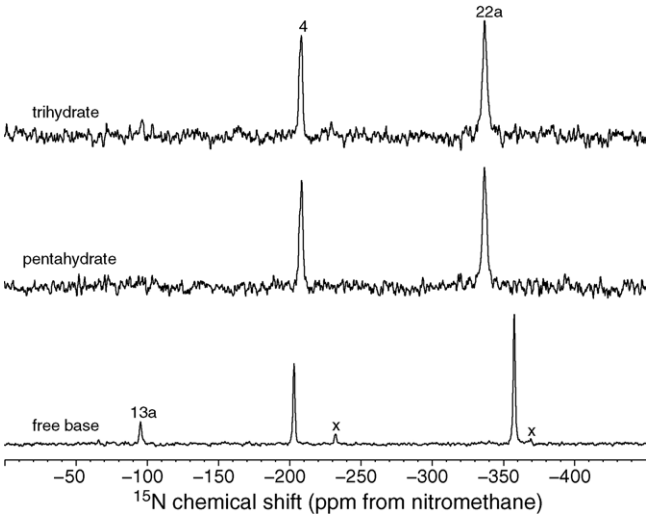


Fig. 8. ^{15}N CP-MAS spectra of topotecan HCl Form II trihydrate (top) and pentahydrate (middle) at 25 °C. Assignments are numbered in reference to the structure of topotecan given in the text. The spectra show no discernable differences, indicating that the loosely held water is probably not in the vicinity of N4 or N22a. No signal is observed from N13a in the topotecan hydrochloride spectra (it is expected at approximately −100 ppm), probably because of weaker cross-polarization for this site and higher chemical shift anisotropy. The loss of signal could also be caused by splitting or broadening due to non-equivalent sites, or insufficient ^{1}H T_1 relaxation. The spectrum of a semi-crystalline form of topotecan free base is shown at the bottom for comparison. A clear deshielding shift of the N22a indicates protonation at this position in the salt.

to some of these sites, most notably C17 and C20. These sites are structural neighbors of C21, the site which showed the most distinctive shifts in Fig. 6. This altered dephasing behavior helps confirm that the lactone portion of the molecule is involved with the channel water.

The results of ^{15}N CP-MAS experiments are shown in Fig. 8. The spectra show no discernable differences with the change in hydration state, indicating that the water in the channels is not able to strongly affect either N4 or N22a. No change was observed in the deuterated pentahydrate ^{15}N spectrum (data not shown). This is consistent with the ^{13}C results, and suggests that although N22a is protonated in the hydrochloride salt, it does not interact with the loosely bound water, although the strongly bound lattice water might be in its vicinity. Unfortunately, the signal from N13a (a possible hydrogen-bonding site) is too weak to be observed in these spectra, most likely because of poor cross-polarization dynamics to this nucleus. Only a single proton, H12, is close enough to N13a to be an intramolecular source of polarization. This site is also expected to have a large shielding anisotropy, which will reduce signal in the centerband at this MAS spinning rate. Insufficient ^{1}H T_1 relaxation could also play a lesser role in the weak signal for this site, as a relatively fast experiment repetition rate (5 s) is needed to acquire this data in a reasonable time. The spectrum of a semi-crystalline form of topotecan free base is also shown in Fig. 8 for comparison. The strong deshielding shift of the N22a signal is consistent with protonation and ionization at this site, as expected from the structure of the molecule.

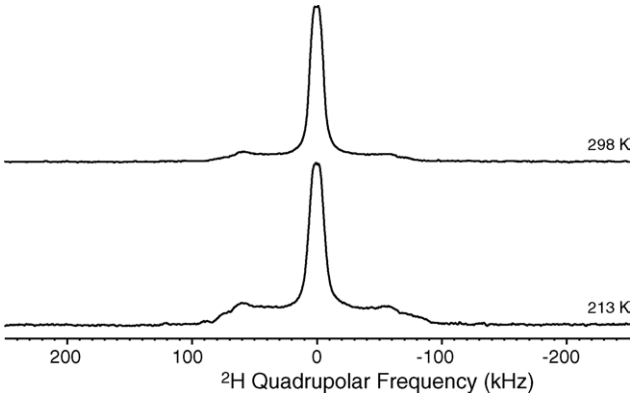


Fig. 9. ^{2}H static quadrupolar echo NMR spectra of D_2O vapor-exchanged topotecan HCl Form II pentahydrate, obtained at 298 and 213 K. The relative invariance of the spectra to temperature along with the narrowed ∼15 kHz ^{2}H lineshape at the center of the powder pattern suggests rapid motion of the vapor-exchanged D_2O in the crystalline lattice, consistent with loosely bound water in channels. The broader component observed in the spectra could be caused by tightly bound ("lattice") water, or by direct exchange of labile deuterons from the D_2O into the protic H_2O or the exchangeable sites on the molecule.

The ^{2}H NMR analysis of the D_2O-exchanged pentahydrate sample offers a direct view of the channel water environment in Form II. The spectra, shown in Fig. 9, are a composite of at least two first-order quadrupolar powder patterns. The motionally narrowed lineshape at the center of the pattern (∼12 kHz full width at half height) is ascribed to rapidly moving D_2O molecules in the lattice. A broadened (∼150 kHz) and less intense pattern is consistent with tightly held water or an exchanged site on the topotecan molecule. The broadened pattern could arise from certain variable water molecules becoming locked into regions of the crystal structure. It could also indicate that deuteron exchange can occur between channel and lattice water, or between channel water and labile sites on the molecule. A minor decrease in the intensity of the central line and an increase in width (to ∼14 kHz) are observed as the temperature is lowered and the thermal mobility of the water is reduced. Similar effects have been noted in the ^{2}H spectra of a cobalt complex exhibiting water tunnels [31].

The combined results of the solid-state NMR study indicate that it is sensitive enough to detect differences in hydration state and is especially useful for the study of topotecan HCl. Even without a crystal structure, the NMR data allows for some inferences to be drawn about the location of the channels. The lactone portion of the molecule shows the most distinctive effects with changes in hydration state and water isotope, and is likely to be involved in holding the 2 mol of channel water. The ester signal (C21) shows an additional site engaged in hydrogen bonding as the hydration state increases. Other hydrogen-bond donors and acceptors (the pyridyl carbons and phenol groups) also show effects both in the spectra and in their dipolar dephasing behavior, suggesting that hydration channels border the lactone, the pyridyl ring, and parts of the phenol/amine region, and that the channel water is intricately tied into the lattice despite being quite mobile (as seen by the ^{2}H spectra). Interpretation of the changes seen in the NMR spectra provides some insight into the intricate role of channel water in the lattice. However, given the unknown packing for this crystal form, the structure of the channels cannot be understood in more detail without a successful single-crystal X-ray diffraction analysis.

4. Conclusions

The structure and variable hydration state behavior was characterized for a stable form of topotecan HCl selected for development. Hydration, vapor sorption studies, and thermal analysis of this form indicate that it is formally a pentahydrate, with two of the five waters being weakly bound in the crystalline lattice. PXRD shows that the loss of these two waters is reversible and does not detectably affect the crystal structure. The loss of the remaining tightly bound water results in collapse of the crystal lattice. ^{13}C and ^{15}N solid-state NMR experiments indicate that two loosely bound moles of water are associated with specific functional groups in the topotecan molecule, especially in the vicinity of the lactone group and the cyclic amide, along with other potential hydrogen-bonding sites. The small changes observed in the ^{13}C NMR spectra upon changing the hydration state were confirmed by re-hydration with D_2O, which caused additional minor spectral effects. In addition, ^{2}H wideline NMR was found to be a promising technique for investigation of channel hydrates, as the samples can be readily prepared, and the data offers insights into the weak bonding and motion of channel water contained in the crystal structure. Solid-state exchange between loosely and tightly bound water molecules or other labile sites on the host molecule may also be directly studied. It is concluded that characterization of crystalline pharmaceutical channel hydrates by a combined approach including solid-state NMR can yield useful structural information even in the absence of single-crystal X-ray data.

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