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Solid-state insight into the action of a pharmaceutical solvate: structural, thermal and dissolution analysis of indinavir sulfate ethanolate

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Abstract

The crystal structure of indinavir sulfate, a pharmaceutical administered as an ethanol solvate, is presented, revealing a unique channel/ionic solvate structure to be characteristic of the compound. The properties of the material with regard to thermal treatment and water adsorption follow closely from the structure. The *in situ* amorphization of the pharmaceutical upon contacting liquid water is observed and highlights the unique dissolution enhancement of marketing the crystalline solvate dosage. Through survey of published crystal structures, an ambiguous sulfate/bisulfate ionization state is also observed in the crystal, which challenges the general understanding of the pharmaceutical. This study provides a solid-state insight into the function of a special multicomponent crystalline pharmaceutical form.

Keywords

Crystal structure; solvates; desolvation; amorphism; salts

Introduction

Active pharmaceutical ingredients (APIs) are commonly developed into dosages as crystalline solid forms.¹ Single component forms, those that only contain the drug molecules, are commonly used although APIs can also be administered as multicomponent solid forms such as salts, cocrystals and solvates to obtain optimal properties.² The formation of solvates is commonly observed during solution-based crystallization of APIs in various media including water and organic solvents.^{3–4} However, solvates besides hydrates are not frequently administered in pharmaceutical products, mainly due to concerns about

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Supplementary Material

This article contains supplementary material available from the authors by request or via the Internet.

the toxicity of the solvents.^{5–8} The stability issues associated with using high volatility solvents also demand thorough solid form property studies of these solvates to assess their susceptibility to desolvation.⁹ These analyses are crucial for the validation of the consistency and reliability of the expected efficacy of the solvates. We are aware of only seven pharmaceuticals marketed as solvates: trametinib,¹⁰ dapagliflozin,¹¹ cabazitaxel,¹² darunavir,¹³ doxycycline,¹⁴ indinavir sulfate,¹⁵ and warfarin sodium.¹⁶ Among these pharmaceuticals, indinavir, darunavir and doxycycline are dosed as ethanol solvates (ethanolates).

Indinavir sulfate is used as an inhibitor of HIV protease, and is marketed by Merck as Crixivan (Figure 1a).¹⁷ It is the only pharmaceutical that is formulated simultaneously as a sulfate salt and an ethanol solvate, due to its superior solubility (more than 25,000 times higher) and oral pharmacokinetics compared to the neutral monohydrate form.^{18–19} The solvate form has also demonstrated satisfactory shelf stability as long as two years.²⁰ However, the crystal structure of indinavir sulfate ethanolate has not been reported so the solid-state basis for these properties is unknown. Herein, we report the crystal structure of indinavir sulfate ethanolate and interpret the structural data to enable better understanding of the physicochemical properties observed for this solid form. The ionization state, which is more complex than previously thought, is determined and interpreted in the context of data culled from the Cambridge Structural Database (CSD) on structurally related compounds. Thermal stability of the pharmaceutical is evaluated by relating the thermal analysis of the desolvation process to the bonding interactions and packing arrangements observed in the crystal structure. Finally, a chemical basis for the known amorphization of this solid form is proposed.

Methods

Single Crystal X-Ray Diffraction

Colorless plates of indinavir sulfate ethanolate (98%, Cayman Chemical Co., Ann Arbor, MI) were grown by completely dissolving 10.0 mg of the compound in 1.0 ml of ethanol at 80 °C and cooling to room temperature. The crystals were collected and immediately analyzed without drying. A crystal of dimensions 0.17 × 0.09 × 0.02 mm was mounted on a Rigaku AFC10K Saturn 944+ CCD-based X-ray diffractometer equipped with a low temperature device and Micromax-007HF Cu-target micro-focus rotating anode ($\lambda = 1.54187 \text{ \AA}$) operated at 1.2 kW power (40 kV, 30 mA). The detailed structural analysis procedure is included in the Supplementary Material.

The Cambridge Structural Database (CSD) Survey

The CSD searches employed ConQuest version 1.18.²¹ Pyridine/pyridinium and sulfate/bisulfate moieties were drawn and searched with the following filters used as criteria: the hits must have determined 3D coordinates and a R factor <0.05 with no disorder or errors. Organometallic structures, polymeric structures and structures determined from powder X-ray diffraction (PXRD) were also excluded from the results.

Results and Discussion

Ionization State Analysis

According to the patent and the analysis published by Merck, the chemical composition of the pharmaceutical Crixivan is indinavir monosulfate ethanolate.^{17, 22} The molecule contains three basic nitrogen atoms (two tertiary amine nitrogen atoms and one pyridine) (Figure 1b) capable of accepting protons from the incorporated sulfuric acid. As illustrated in the determined crystal structure of indinavir sulfate, one of the two tertiary amine nitrogen atoms is clearly protonated, whereas the case associated with the protonation of the pyridine nitrogen is more complicated. It is often observed that in the solid state when an acid-base pair is present, a pK_a value ($pK_a = pK_a$ of the protonated base – pK_a of the acid) greater than 3 will result in salt formation;^{23–24} however, proton transfer is also dependent on the specific molecular environment in the crystal structure.^{25–27} Based on the empirical rule, the pK_a of a pyridine/bisulfate pair is equal to ~3.2,²⁸ which predicts the favorable formation of a pyridinium cation and sulfate anion. It should be noted that pharmaceuticals administered as bisulfate are relatively rare. The FDA Orange Book Database, as of June 2017, shows the presence of 41 approved sulfate drug products with therapeutic equivalence evaluations, but only one bisulfate drug, clopidogrel bisulfate.²⁹ However, the crystal structure determination of indinavir demonstrates the presence of a more ambiguous ionization state between sulfate and bisulfate. The proton placement derived directly from the diffraction data shows the presence of a bisulfate OH bond, albeit with an unusually long O-H bond distance of 1.13(7) Å.

Analysis of the CSD was undertaken to elucidate the ambiguous proton assignment by assessing the resemblance between the structural features of the determined indinavir structure and those of the existing structures in the database. An unconventional bisulfate O-S bonding distance (1.523(3) Å) and pyridine C-N-C angle (119.2(4)°) in the determined structure corresponding to an intermediate state between sulfate ion and bisulfate ion were observed. As illustrated in Figure 2a, the C-N-C angle in the determined structure is smaller than the angles of 95.7° of the pyridinium ions and greater than the angles of 95.9° of the neutral pyridines in the CSD. Similarly, the O-S bond distance is shorter than 89.5% of the protonated O-S bond distances and longer than 96.4% of the neutral O-S bond distances in the CSD (Figure 2b). It is therefore demonstrated that the proton is shared by both the pyridine and the sulfate ion instead of completely bonded to either. In other words, the actual ionization state is an intermediate between “indinavir sulfate” and “indinavir bisulfate”. Similar cases of ambiguous ionization state have been reported.^{30–31}

Physical Property Analyses

As the only pharmaceutical marketed as a sulfate/bisulfate salt and ethanol solvate, Crixivan exhibits complicated thermal and water sorption properties that are closely related to its crystal structure. The determined crystal structure reveals a hydrogen bonding network constructed around the sulfate/bisulfate ion (Figure 1b). The indinavir chains are bridged by the sulfate/bisulfate ions and the ethanol molecules. Each sulfate/bisulfate ion is connected to three indinavir molecules through hydrogen bonding interactions: sulfate/bisulfate to pyridine (O-H···N), sulfate/bisulfate to the hydroxyl group on the five-membered ring (O-

H $\cdots$ O) and sulfate/bisulfate to the secondary amine group (N-H $\cdots$ O). Additionally, each sulfate/bisulfate is tethered to two ethanol molecules through sulfate/bisulfate-hydroxyl hydrogen bonds (O-H $\cdots$ O and O-H $\cdots$ O). The structure was best modeled with a 1.0:1.5 indinavir:ethanol stoichiometry. One of the ethanol molecules with full occupancy is disordered in two positions (only one position is shown in Figure 1b) and provides additional bridging interactions by forming a hydrogen bond with the protonated tertiary amine group (H-N $^+$ $\cdots$ O). The other ethanol molecule possesses 50% occupancy and acts as a terminal ligand bonded to the sulfate/bisulfate ion. These hydrogen bonding interactions associated with ethanol molecules resemble those of ion-coordinated solvates, where high desolvation temperatures are expected. The indinavir molecules form zigzag chains which are intercalated by sulfate/bisulfate ions. The chains orient around the 2_1 axis and form a helical ladder structure (Figure 3a). The ethanol molecules, on the other hand, form solvent channels that extend in parallel with the helices along the b -axis (Figure 3b). The formation of these channels makes the ethanol molecules susceptible to diffusion out of the crystal lattice (Figure 3c).

Thermogravimetric analysis (TGA) of the indinavir sulfate/bisulfate ethanolate shows a broad mass loss of 4.91% occurring from 25 °C to ~145 °C, indicating a 0.8:1 molar ratio of ethanol to indinavir. This is supported by differential scanning calorimetry (DSC) measurements, showing a sharp endothermic peak with a T at 147 °C (Figure S2).³² max Nuclear magnetic resonance (NMR) analysis shows the crystals to contain an ethanol to indinavir ratio of 0.8:1 as well, but the previously discussed crystal structure of freshly harvested sample indicates much more solvent to be present, at a 1.5:1 ethanol to indinavir ratio (Figure S3). The discrepancy is attributed to difficulties of accurately modeling the disordered ethanol molecules in the structure and the often non-stoichiometric nature of channel solvates. In summary, these results show that the ethanolate crystal is characteristic of both channel solvates (non-stoichiometric, broad temperature loss) and ion-coordinated solvates (desolvation occurring at a much higher temperature than the boiling point of the solvent).

Temperature-dependent PXRD of indinavir sulfate/bisulfate ethanolate was carried out to monitor phase changes during the desolvation process. Figure 4 shows a gradual loss of peaks in the powder patterns, indicating a continuous decrease of crystallinity as the temperature increases, with desolvation of the sample leading to the formation of an amorphous product. This observation deviates from the typical characteristics of channel solvates where isostructural desolvated products are often observed. Potential phase transformations of the amorphous, desolvated form were also investigated. This form was converted back to the ethanolate by cooling crystallization in ethanol, whereas it failed to be converted to other crystalline forms through either cooling or evaporation of saturated solutions or slurring of solid in five other solvents (water, hexane, dichloromethane, acetone, and ethyl acetate).

The hygroscopicity of indinavir sulfate/bisulfate ethanolate was also investigated in relation to the crystal structure. It has been reported that the pharmaceutical transforms to the amorphous form at elevated humidity.¹⁹ We hypothesize that this result is correlated with the characteristics of the channel/ionic solvate. The solvent channels in the structure enable

water molecules to penetrate the lattice and interrupt the intermolecular interactions around the ethanol molecules, leading to desolvation and amorphization of the bulk material. This behavior contrasts with channel solvates that allow water to simply replace organic solvent within the channels. For this case, water-ethanol interactions may be providing a strong driving force for desolvation instead of solvent exchange. The indinavir solid is then left without a suitable channel-structuring solvent and the amorphization ensues. In a similar manner, amorphization of the ethanolate crystal occurs upon contact with liquid water, as evidenced by PXRD analysis. This *in situ* amorphization inhibits the characterization of equilibrium solubility of the crystalline form and explains the equality of the measured dissolution rates of the crystalline ethanolate and the desolvated sample (Figure S4). It is a unique property of this crystal form, as amorphous pharmaceutical forms are highly desired for improving dissolution, and extensive measures often must be taken to achieve this result. Here this same performance enhancement is achieved with the advantages of a crystalline dosage.

In conclusion, the newly determined crystal structure of indinavir sulfate/bisulfate provides a mechanistic understanding of the solid-state properties of the pharmaceutical. The structural analysis reveals an ambiguous assignment of proton location where a proton is shared between the pyridine and the sulfate ion, demonstrating an intermediate ionization state between indinavir sulfate and bisulfate that challenges the currently accepted nomenclature of the pharmaceutical. The thermal stability and hygroscopicity of the ethanolate are closely related to the structural features of the crystal. Both thermal desolvation and contact with water cause collapse of the crystal lattice, resulting in an amorphous delivered form. This report highlights the insights available from structural characterization in order to thoroughly understand the intertwined physical properties in complex multicomponent solid forms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Abbreviations used:

API	active pharmaceutical ingredient
CSD	Cambridge structural database
TGA	thermogravimetric analysis
DSC	differential scanning calorimetry
NMR	nuclear magnetic resonance
PXRD	powder X-ray diffraction

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Figure 1.

a) Chemical structure of indinavir sulfate ethanolate (Crixivan). b) Illustration of hydrogen bonding interactions in the crystal structure of indinavir sulfate/bisulfate ethanolate revolving around the sulfate/bisulfate ion; only fragments of indinavir molecules are shown. White = hydrogen, grey = carbon, blue = nitrogen, red = oxygen, yellow = sulfur.

Figure 2. Statistical analysis of C-N-C bond angles in structures containing pyridine/pyridinium (a) and OS bond distances (b) in structures containing sulfate/bisulfate collected from the CSD.

Figure 3.

Crystal structure overview of indinavir sulfate/bisulfate ethanolate. a) The helical chains intercalated by sulfate/bisulfate ions along *b*-axis. Both ethanol molecules are presented as whole occupancy with the disordered parts omitted for clarity. Blue = indinavir molecule, white = hydrogen, black = carbon, red = oxygen, yellow = sulfur. b) Illustration of the solvent channel along *b*-axis, ethanol molecules are shown in space-filling style. c) View of the void space in the channels with indinavir sulfate/bisulfate shown in space-filling style with ethanol omitted.

Figure 4.
Temperature-dependent PXRD patterns of indinavir sulfate/bisulfate ethanolate.