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Water-mediated phase transformations of posaconazole: An intricate jungle of crystal forms

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ABSTRACT

Posaconazole is a broad-spectrum antifungal agent exhibiting rich polymorphism. Up to now, a total of fourteen different crystal forms have been reported, sometimes with an ambiguous nomenclature, but less is known about their properties and stability relationships. Investigating the solid-state of a drug compound is essential to identify the most stable form under working conditions and to prevent the risk of undesired solid-phase transformations under processing and storage.

In this paper, we study posaconazole polymorphism by providing a description of its polymorphs, hydrates, and solvates. Powder X-ray diffraction (PXRD), dynamic vapor sorption (DVS), spectroscopic and thermal techniques were employed to characterize the different forms.

In addition, the solid-phase transformations of posaconazole in aqueous suspensions were studied by means of Raman microscopy. Surprisingly, we found that Form S, the crystal form contained in the marketed oral suspension, is not the most stable form in water. Form S readily converts to a more stable hydrate, i.e. Form A, after storage in water for two weeks. In the commercial oral formulation the conversion between the two forms is prevented by the presence of polysorbate 80. Such insights into the stabilizing excipient effects beyond particle dispersion are critical to formulators.

1. Introduction

Polymorphism is the ability of single molecules or identical chemical compositions to crystallize in different crystal arrangements (McCrone, 1965; Bernstein, 2002). In the pharmaceutical industry, the interest in polymorphism of molecular crystals has grown significantly after the "Norvir" case. Norvir® was the name of a commercial semisolid formulation in a hard gelatine capsule, employed in the treatment of HIV infection, based on a nearly saturated hydroalcoholic solution of the crystalline Form I of ritonavir. The production of the formulation was jeopardized by the unexpected precipitation of a new, poorly soluble polymorph of the drug, Form II, in 1998 (Bučar et al., 2015). Due to the higher stability of Form II, all attempts to reproduce Form I thereafter failed, and the formulation had to be withdrawn from the market (Chemburkar et al., 2000).

The event illustrated the importance of carefully investigating the solid-state of the Active Pharmaceutical Ingredient (API) to prevent the risk of undesired solid-phase transformations under processing and storage. Indeed, polymorphic forms differ in terms of stability and physical properties: the less stable (metastable) polymorphs, due to the higher Gibbs free energy, may exhibit advantageous solubility, dissolution rate and, thus, oral bioavailability but tend to convert to the thermodynamically most stable form (Coquerel, 2018). However, in some cases practically isoenergetic drug polymorphs have been observed (Bauer-Brandl et al., 1999) for which other physico-chemical properties, such as processability, are the criteria for preference (Bauer-Brandl, 1996).

In addition, solvent molecules can be incorporated in the crystal lattice of drug substances and a most important subclass of such solvates are hydrates. Hydrates of the API are typically less soluble in aqueous

Abbreviations: API, Active pharmaceutical ingredient; ASDs, Amorphous solid dispersions; PSZ, Posaconazole; AUC, Area under the curve; DCM, dichloromethane; DMF, N,N-dimethylformamide; PXRD, Powder X-ray diffraction; TG-FTIR, Thermogravimetry coupled with Fourier Transform Infrared Spectroscopy; DSC, Differential scanning calorimetry; DVS, Dynamic vapor sorption; VT-PXRD, Variable-temperature Powder X-ray diffraction; HPMC, Hydroxypropyl methylcellulose.

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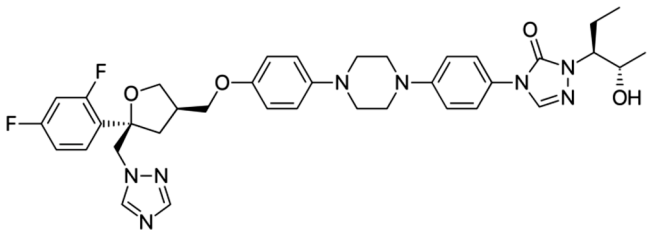

Fig. 1. Molecular structure of posaconazole.

media than the anhydrous (non-solvated) form (Shefter and Higuchi, 1963) and can be produced by interaction with water vapor (e.g., exposure to ambient atmosphere) or liquid water (e.g., wet grinding) during processing or storage of the final product.

Since most of newly developed drug compounds are intrinsically poorly water-soluble, processing into solid forms able to generate supersaturation after oral formulation administration is commonly employed. Cocrystals (Childs et al., 2013), salts, nanocrystals, and amorphous solid dispersions (ASDs) (Almeida e Sousa et al., 2016) are promising approaches adopted to solve this biopharmaceutical problem.

Weakly basic drugs are of particular importance in this context due to their intrinsic tendency to induce supersaturation in vivo. However, supersaturated systems are unstable and eventually result in phase separation (liquid-liquid, liquid-glass) or drug precipitation (Hsieh et al. 2012; Tho et al., 2010; Bevernage et al, 2013). Precipitation of weakly basic compounds and its impact on bioavailability were studied in detail, e.g. for dipyridamole (Kostewicz et al., 2004; Eriksen et al., 2020) and for antifungal agents, such as itraconazole (Kourentas et al., 2016, Nunes et al, 2023a; Nunes et al., 2023b) and posaconazole (Hens et al., 2016; Hens et al., 2018; Holzem et al., 2022; Holzem et al., 2023).

Understanding the precipitation behavior and the impact on oral absorption of weakly basic drug substances is critical for the design of products with optimized bioavailability. Knowing the solid-state behavior of the API is mandatory for achieving this goal because polymorphic transition and hydrate formation can occur during drug dissolution and precipitation. Two mechanisms are usually involved in the phase conversion: i) solid-solid phase transformation, in which the molecules rearrange in the crystal lattice while remaining in the solid state and ii) solvent-mediated phase transformation where the metastable form dissolves and creates the necessary supersaturation for nucleation and growth of a more stable anhydrous or hydrated crystal (Cardew and Davey, 1985; Rodríguez-Hornedo et al., 1992). Polymeric excipients may be added to prevent the crystallization of amorphous material (Edueng et al., 2017) or the conversion from anhydrous to hydrated form (Kirchmeyer et al. 2016; Paisana et al., 2017). Indeed, polymers may adsorb on the surface of the existing solid preventing interaction with dissolved molecules and inhibiting nucleation.

In the present work, the importance of investigating the solid-state landscape of an API is exemplified by posaconazole (PSZ), a broad-spectrum antifungal agent employed in the treatment of yeast and mold infections (Fig. 1). The drug belongs to the Biopharmaceutics Classification System (BCS) class II, as demonstrated by the low water solubility (<1 μg/mL; Duong et al., 2022) and high lipophilicity value (log P= 4.6; Walravens et al., 2011). Oral formulations available on the market comprise an oral suspension (Noxafil® 40 mg/mL) and a gastro-resistant delayed-release tablet (Noxafil® tablet 100 mg).

Up to now, a total of fourteen different crystal forms of posaconazole have been reported, in some cases with an ambiguous, even confusing, nomenclature. The adjectives ambiguous and confusing are used in this case because roman numbers (Form I, I′, II, III, IV, V), latin letters (Form A, S, Y, N) and a combination of the two (Form II-S) were used to name posaconazole crystal forms. In addition, the same form was reported with different names (e.g. Form III and methanol solvate) or the same name was used for different solid forms (e.g. Form II was associated to Form Y even if it is a poorly crystalline Form A). The crystal forms are listed and described in Table 1 together with three new posaconazole solvates. Since most of the forms have been described in patent applications, little is known about their characterization. Only recently have new studies started elucidating the polymorphism of posaconazole and its crystallization behavior (Din et al., 2020). In addition, Din et al. (2020) investigated the crystallization kinetics and stability of amorphous posaconazole. The structural characterization of two anhydrous polymorphs, Form I and Form Y, and of two solvates, methanol (Form III) and dioxane-water solvate, have been described (McQusiton et al., 2019; Yao et al., 2022; Du et al., 2023). According to the current knowledge, Form I is the thermodynamically stable polymorph of posaconazole at ambient temperature and low humidity. Form I was also reported to be used in the manufacturing of the marketed oral suspension (European Medicines Agency, 2019). However, Lykouras et al. (2020) demonstrated that a different crystal form, Form S, was actually present in the suspension. Indeed, Form I undergoes a phase transformation to Form S in water (Lykouras et al., 2023). The crystal form was suggested to be a channel hydrate of posaconazole even if a definitive proof of its nature was not provided.

Despite the advances made in the field of posaconazole polymorphism, the phase behavior of the drug is still not fully understood.

Table 1
List of posaconazole polymorphs, hydrates and solvates, description of the forms and final identification. The forms listed in bold were successfully reproduced within this work.

Crystal Form	References	Description	Proposed name
Form I	Andrews et al. 1999; McQuinston et al. 2019	Stable anhydrous form	**Form I**
Form I′	Yao et al. 2022; this work	Anhydrous Form, enantiotropically related to Form I	**Form I′**
Form Y or II	(Y) Wieser et al. 2013; Din et al. 2020; (II) Yao et al. 2022	Anhydrous form	**Form II**
Form II	Andrews et al. 1999	Poorly crystalline Form A	-
Form III	Andrews et al. 1999; McQuinston et al. 2019; this work	Crystalline form with channels able to accommodate methanol (m) and/or water (w)	**Form $III_{m/w}$**
Form IV	Wieser et al. 2010	Channel hydrate able to accommodate 1.5 mol of water	Form IV
Form V	Gharpure et al. 2011	-	Form V
Form A	Badone et al. 2015	Channel hydrate able to accommodate up to 3 mol of water	**Form A**
Form II-S	Wieser et al. 2012	Channel hydrate able to accommodate up to 2 mol of water	**Form II-S**
Form S	Satyanarayana et al. 2013; Lykouras et al. 2020; Lykouras et al. 2023	Likely a channel hydrate	**Form S**
Form N	Satyanarayana et al. 2013	Mixture of Form II-S and Form S	-
Water-dioxane	McQuinston et al. 2019	PSZ:water:dioxane stoichiometry of 1:1:1	**Water-dioxane solvate**
Methanol	McQuinston et al. 2019	Crystalline form with channels able to accommodate methanol (m); see Form III	**Form III_m**
Isopropanol	Satyanarayana et al. 2013	Mixture of Form I and isopropanol solvate	**Isopropanol Solvate**
Pyridine	This work	Pyridine solvate	**Pyridine Solvate**
DCM	This work	DCM solvate	**DCM Solvate**
DMF	This work	DMF solvate	**DMF Solvate**

Conducting stability studies of the hydrate forms may give information about the phase transitions in water and determine whether Form S, present in the oral suspension, is the most stable form of posaconazole in aqueous media. In addition, profound understanding of the polymorphism is required while searching for new multicomponent crystal forms of the compounds, as we learned from the discovery of new posaconazole cocrystals (Guidetti et al., 2023).

The present paper attempts to shed light onto the confusing topic of posaconazole solid-state forms. Characterization of anhydrous, hydrate and solvate forms of the drug substance was conducted using spectroscopic, thermal, and dynamic vapor sorption techniques. For the first time, a full categorization (Table 1) and a comprehensive description of each form is provided. The collected data was used to investigate the phase conversion of posaconazole crystal forms in water and is reported in the discussion section.

2. Materials and methods

2.1. Materials

Posaconazole (PSZ) was purchased from BOC Sciences (Shirley, NY) and used without further purification. Powder X-ray diffraction of the purchased PSZ showed that the sample was mainly amorphous with the presence of low intensity peaks that are attributable to posaconazole Form I (supplementary material, Figure S2). The chemical identity of PSZ was confirmed by proton nuclear magnetic resonance (^{1}H NMR). Organic solvents were purchased from Merck & Cie, (Schaffhausen, Switzerland). Ultrapure Milli-Q water (resistivity 18.2 MΩ·cm at 25 °C; total organic carbon (TOC) $\leq$ 5 ppb) was prepared in the laboratory and used in the performed experiments.

Noxafil® 40 mg/mL suspension (Merck Sharp & Dohme B.V., Haarlem, Netherlands) was purchased from a local pharmacy.

2.2. Preparation of PSZ polymorphs

The following subsections describe the approaches used to prepare the solid forms of posaconazole studied within this work. As described in Section 3.4, Forms IV and V could not successfully be reproduced. Form I′ was prepared in situ by heating Form I as described in Section 3.1.1. The names of the crystal forms in the captions are according to the nomenclature proposed in Table 1.

2.2.1. Preparation of PSZ Form I

Approximately 60 mg of the supplied mostly amorphous PSZ was added to ethanol (2 mL) and the system was stirred with a magnetic bar for 1 day at RT. A white powder was isolated after vacuum filtration (0.2 μm PTFE filter).

2.2.2. Preparation of PSZ Form II

This form was previously reported as Form Y. Following Wieser et al. (2013) procedure, approximately 20 mg of PSZ Form III was added to a glass vial preheated to 140 °C. The crystalline powder was left at 140 °C under N_2 flow (50 mL/min) for 15 minutes and was subsequently cooled to RT by letting the system stand under ambient conditions for one hour.

2.2.3. Preparation of PSZ Form III

Approximately 70 mg of the supplied mostly amorphous PSZ was added to methanol (2 mL) and the system was stirred with a magnetic bar for 2 days at RT. A white powder was isolated after vacuum filtration (0.2 μm PTFE filter).

2.2.4. Preparation of PSZ Form A

In a new preparation method, approximately 50 mg of either the supplied mostly amorphous PSZ or PSZ Form I was suspended in a THF-H_2O 50 % v/v solution (2 mL). The system was stirred with a magnetic bar for two days at RT. Then, the suspension was vacuum filtered using a 0.2 μm PTFE filter; a white powder was isolated.

2.2.5. Preparation of PSZ Form II-S

In a new preparation method, about 60 mg of either amorphous PSZ or PSZ Form I was suspended in an acetone-H_2O 50 % v/v solution (3 mL). The system was stirred with a magnetic bar for 5 days at RT. Then, the suspension was vacuum filtered using a 0.2 μm PTFE filter; a white powder was isolated.

2.2.6. Preparation of PSZ Form S

The crystalline form S was isolated from the marketed Noxafil® oral suspension following the procedure reported by Lykouras et al. (2020): about 4 mL of oral suspension was added to a 10 mL plastic test tube; the suspension was centrifugated at 8000 rpm for 23 minutes at 25 °C; the supernatant was withdrawn, and the wet phase at the bottom was measured by PXRD. In addition, Form S was also prepared by adding approximately 40 mg of PSZ Form I to water (2 mL). The system was sonicated in a sonicating bath for 1 minute and was then stirred with a magnetic bar for 1 day at RT. The suspension was filtered gravimetrically with the use of a centrifuge (5000 rpm, 5 min, 23 °C) and a 0.2 μm PTFE filter. A wet, white powder was isolated and was directly measured by PXRD.

2.2.7. Preparation of PSZ 1,4-dioxane water solvate

In a new preparation method, approximately 40 mg of the supplied mostly amorphous PSZ was suspended in a 1,4-dioxane:H_2O 50 % v/v solution (2 mL). The system was stirred with a magnetic bar for 4 days at RT. Then, the suspension was filtered gravimetrically with the use of a centrifuge (5000 rpm, 5 min, 23 °C) and a 0.2 μm PTFE filter.

2.2.8. Preparation PSZ isopropanol solvate

In a new preparation method, approximately 75 mg of the supplied mostly amorphous PSZ was suspended in isopropanol (2 mL). The system was stirred with a magnetic bar at RT for 1 week. Then, the suspension was vacuum filtered with the use of a 0.2 μm PTFE filter; a white powder was obtained.

2.2.9. Preparation of PSZ pyridine solvate

Approximately 60 mg of PSZ Form I was added to a glass vial and dissolved in pyridine (0.4 mL). The solution was heated up to 35 °C and was evaporated under N_2 flow (50 mL/min) overnight. The formation of an off-white powder was observed the following day.

2.2.10. Preparation of PSZ DCM solvate

The crystalline compound was isolated in an attempt to obtain Form V following the procedure reported by Gharpure et al. (2011). Approximately 150 mg of PSZ Form I was dissolved in 4 ml dichloromethane (DCM). A clear solution was obtained, and the system was filtered using a 0.2 μm PTFE filter. The solution was evaporated using a rotary evaporator (35 °C bath temperature) starting from a pressure of 650 mmHg and decreasing it gradually to 100 mmHg. A white powder was obtained after complete removal of solvent.

2.2.11. Preparation of PSZ DMF solvate

Approximately 15 mg of the supplied mostly amorphous PSZ was dissolved in 150 μL of N,N-dimethylformamide (DMF). The solvent was slowly evaporated at RT, eventually resulting in the isolation of white powder.

2.3. Crystal form conversion in aqueous media

2.3.1. Competitive slurry between Form A and Form II-S

Approximately 30 mg of PSZ Form A and 30 mg of PSZ Form II-S were mixed in a mortar. The mixture was suspended in water (2 mL) and was sonicated for 1 minute. The suspension was stirred with a magnetic bar for 10 days at RT and was then filtered gravimetrically in a

centrifuge (5000 rpm, 5 min, 25 °C) using a 0.22-μm PTFE filter; The isolated white powder was confirmed to be Form A by PXRD (Section 3.5.1).

2.3.2. Competitive slurry between Form A and Form S

Approximately 20 mg of PSZ Form I was added to water (2 mL) and was sonicated for 1 minute. Then, around 20 mg of PSZ Form A were added to the suspension and the system was stirred with a magnetic bar for 10 days at RT. The system was filtered gravimetrically in the centrifuge (5000 rpm, 5 min, 25 °C) using a 0.22-μm PTFE filter; The isolated white powder was confirmed to be Form A by PXRD (Section 3.5.1).

2.3.3. Conversion of Form S in water

Approximately 60 mg of PSZ Form I was added to 2 mL water. The suspension was sonicated for 40 seconds and then left stirring with a magnetic bar at 350 rpm. A few drops of suspension (20 μL) were deposited on a metal slide and measured by Raman microscopy after 2 minutes, 2 hours and weekly for a total of 5 weeks. After 3 weeks, around 1 mL of suspension was filtered with the use of a centrifuge (5000 rpm, 5 min, 23 °C) and a 0.2 μm PTFE filter; The isolated white powder was confirmed to be Form A by PXRD (Section 3.5.2).

2.3.4. Conversion of Form S in polysorbate 80 aqueous solution

Approximately 60 mg of PSZ Form I was added to 1.5 mL of polysorbate 80 (Tween 80) aqueous solution (0.1 mg/mL or 2.5 mg/mL). The procedure reported in Section 2.3.3 was followed to investigate the solid phase. After 3 weeks, the isolated white powder was confirmed to be Form S by PXRD (Section 3.5.3).

2.4. Methods

2.4.1. Powder X-ray diffraction (PXRD)

Diffractograms were collected using a Stoe Stadi P diffractometer (Stoe & Cie. GmbH, Darmstadt, Germany) equipped with a Mythen1K detector operating with Cu-$K_{\alpha,1}$ radiation. The measurements were performed in transmission at a tube voltage of 40 kV and 40 mA tube power. A step size of 0.02° 2θ and a step time of 12 seconds over a 1.5–50.5° 2θ scanning range was applied. For a typical sample preparation about 10–20 mg of sample was placed between two acetate foils and mounted into a Stoe transmission sample holder. The sample was rotated during the measurement. All sample preparation and measurement were done at RT in an ambient air atmosphere.

2.4.2. FT-Raman spectroscopy

FT-Raman spectra were recorded on a Bruker MultiRAM FT-Raman spectrometer (Bruker AG, Fällanden, Switzerland) with a near infrared Nd:YAG laser operating at 1064 nm and a liquid-nitrogen-cooled germanium detector. A total of 64 scans with a resolution of 2 cm^{-1} was accumulated in the range from 50 to 3500 cm^{-1} using a nominal laser power of 100 mW. The samples were prepared by pressing the isolated powder into an aluminum sample holder.

To collect the FT-Raman spectrum of Form S, the following procedure was performed: a suspension of the material was poured in a truncated, 5 mm diameter NMR tube, the tube was centrifuged, and the spectrum was collected on the powder deposited at the bottom of the tube.

2.4.3. Thermogravimetry Coupled with Fourier Transform Infrared Spectroscopy

Thermogravimetry coupled with Fourier Transform Infrared Spectroscopy (TG-FTIR) was performed on a Netzsch Thermo-Microbalance TG 209 (Netzsch, Selb, Germany), which is coupled with a Bruker FT-IR Spectrometer Vector 22. The aluminum crucibles had a (micro) pinhole, and the measurements were carried out under a nitrogen atmosphere and at a heating rate of 10 °C/min over the range 25–250 °C.

2.4.4. Differential scanning calorimetry (DSC)

DSC analyses were carried out with a TA Instruments Q2000 or with a TA Instruments DSC 2500 (TA Instruments, New Castle, Delaware, USA). Approximately 2–3 mg of sample was heated at a rate of 10 or 20 K/min under nitrogen gas flow (50 mL/min). Standard aluminum sample pans that were either closed or perforated with a pinhole were used for the measurements.

2.4.5. Dynamic vapor sorption (DVS)

Most DVS measurements were performed with a DVS Resolution from Surface Measurement Systems Ltd. (London, United Kingdom). The accuracy of the balance is ± 0.1 μg. Water or methanol was employed as the vapor generating solvents in combination with isothermal programs at 298.15 K. About 10–20 mg of sample was put into an aluminum sample pan. In a standard measurement, the sample was allowed to equilibrate at 50 % relative humidity (r.h.). before starting the predefined humidity programs that consists of two cycles of linear humidity ramps at rates of 5 % r.h. per hour from a minimum of 0 % r.h. to a maximum of 95 % r.h. The sample was kept at constant r.h. for 5 hours at the humidity extremes (0 % and 95 % r.h.). The relative humidity profile employed in the DVS measurements is reported in the supplementary material (Figure S1). The DVS performed on samples of Form S was conducted following the same procedure but starting from 95 % r.h.

Some of the standard measurements with water vapor were performed using the same humidity program on an SPS11–100n “Sorptions Prüfsystem” from ProUmid (Ulm, Germany).

2.4.6. Raman microscopy

Samples were analyzed using a Horiba XploRA PLUS confocal Raman microscope from Horiba Jobin Yvon SAS (Villeneuve d’Ascq, France) equipped with a 100 mW 785 nm diode laser for excitation and a Syncerity OE CCD detector. Measurements were carried out with a long working distance 20 × objective, a 50 % or 100 % laser power, a 1200 lines/mm grating, a 100 μm slit and with 30 second accumulation on a measurement range of 200–2000 cm^{-1}. Standard measurements were performed by placing a spatula tip’s worth of the powdered sample on either a quartz or stainless-steel slide. Suspension of posaconazole in water were measured by placing few drops of suspension on a stainless-steel slide and by focusing on small particles of powder; the sample was measured before it was completely dry.

2.4.7. Raman microscopy at variable temperature

The measurements were collected on the Horiba XploRA PLUS confocal Raman microscope using a THMS600 heating and freezing stage equipped with a T95-LinkPad system controller from Linkam Scientific Instruments Ltd. (Redhill, United Kingdom). Measurements were carried out with a 785 nm diode laser and a long working distance 20x objective on a measurement range of 200–2000 cm^{-1}. The sample was sandwiched between two round quartz covers and was heated from 25 °C to 150 °C with a heating rate of 5 K/min. The heating was stopped at selected temperature, and it was waited one minute before collecting the Raman spectrum of the sample. Spectra were collected at 25, 100, 110, 120, 130, 140, 150 °C, and after cooling down the sample to 25 °C.

2.4.8. Powder X-ray diffraction at variable temperature (VT-PXRD)

Diffractograms were collected using a PANalytical X’Pert PRO-MPD diffractometer (Malvern Panalytical Ltd, Malvern, United Kingdom) equipped with a PIXCEL detector operating with Cu-$K_{\alpha,1}$ radiation. The measurements were performed in reflection at a tube voltage of 45 kV and 40 mA tube power, Soller slit: 0.04, Ni-Filter 0.02 mm, Inc Mask Fixed 5 mm (MPD/MRD). A step size of 0.013° 2θ and a step time of 40.8 seconds over a 2–34° 2θ scanning range was applied. The powder sample was measured in a 0.4-mm deep, nickel-coated copper sample holders without any special treatment other than the application of slight pressure to get a flat surface. The sample was measured in a Anton Paar TTK-450 chamber, and an ambient air atmosphere was used. The sample

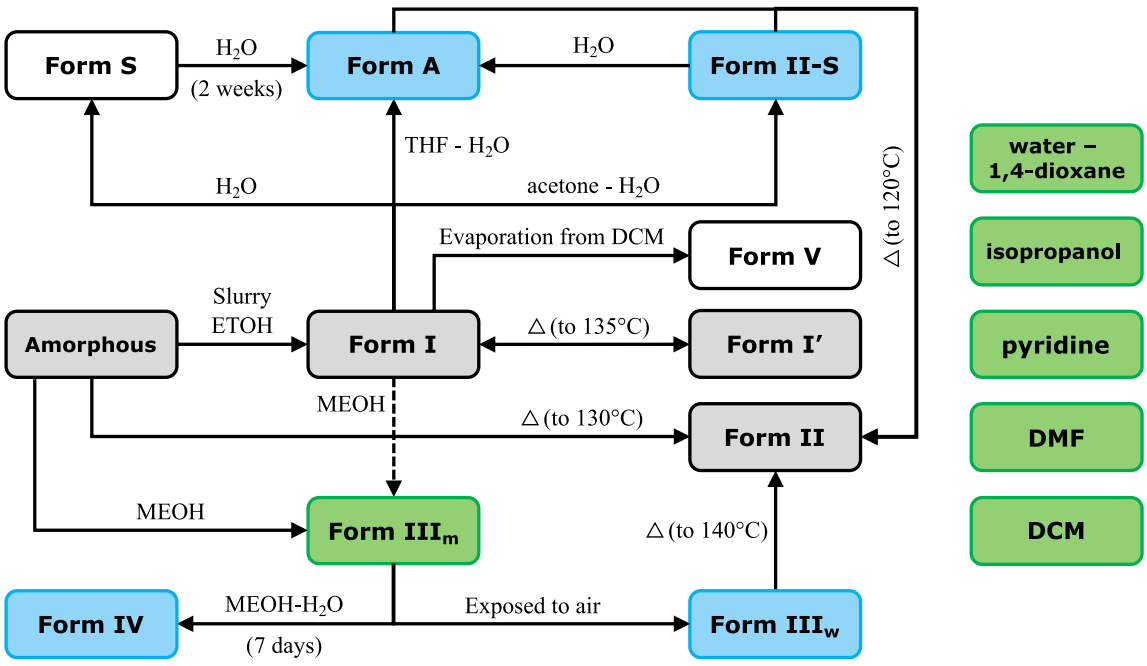

Fig. 2. Diagram representing the conversion of anhydrous (grey) and hydrate (light blue) forms of posaconazole. Forms S and V are reported in white because their nature is still unclear. Solvates other than hydrates are represented in green.

was not rotated during the measurement. All sample preparations and measurements were done at RT in an ambient air atmosphere. The sample was heated at a constant rate of 10 K/min. The heating stopped at preselected temperatures and the temperature was held constant for 5 minutes, then the sample was measured.

3. Results and discussion

Investigating the polymorphic and hydrate forms of an API is an essential part of early pharmaceutical profiling. Such knowledge is crucial for understanding whether the solid form selected for drug development is stable under the conditions it will be exposed to, or whether it would convert into a lower energy form. In the case of posaconazole, several crystal forms have been reported previously in patent applications and in the scientific literature; however, grasping their nature may be difficult due the lack of physical characterization and the complex nomenclature that has evolved over time. Therefore, this section contains a detailed description of the discovered polymorphs, hydrates and solvates of posaconazole (Fig. 2). The categorization of the plethora of forms is presented in Table 1 where the proposed naming scheme reflects the history of the compound in the literature. We merely removed names that correspond to other forms or mixtures thereof because an entirely new nomenclature would be even more confusing. The collected data will be discussed to interpret solid-phase transformations of posaconazole in aqueous media, and to understand why Form S is present in the marketed oral suspension.

X-ray diffraction patterns, FT-Raman spectra, DSC and TG-FTIR thermograms of posaconazole crystal forms are available to the readers in the supplementary material (Figures S2 – S42), they may serve as a guide for the identification of posaconazole polymorphs.

3.1. Description of anhydrous forms

3.1.1. Form I and Form I

To date, Form I is the most stable crystal form of posaconazole at ambient temperature and pressure (298.15 K, 1 bar) and at low humidities. This form can be obtained easily by suspending or recrystallizing amorphous posaconazole in an organic solvent, such as acetonitrile and ethanol; needle-like crystals are usually produced by these methods.

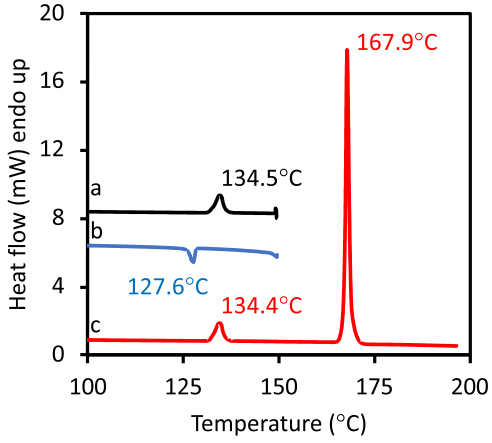

Fig. 3. Three consecutive DSC cycles of posaconazole Form I with a heating/cooling rate of 10 °C/min: a) heating from 25 to 150 °C; b) cooling from 150 to -10 °C; c) heating from -10 °C to 195 °C. For visualization purposes, the axis is displayed from 100 °C because no thermal events occur below 127 °C. The full DSC plot of Form I is reported in the supplementary material (Figure S4) The DSC plots have been shifted along the vertical axis for a better representation.

The thermal behavior of Form I is highlighted in Fig. 3. The DSC thermogram in Fig. 3c shows two endothermic peaks: the first, around 134.5 °C, is related to a phase transition and the second, at 167.9 °C, is due to the melting of the compound. Previous literature considered the small endothermic peak to represent a nematic-like phase transition (Adrjanowicz et al., 2013), which is a conversion from a crystalline phase to a mesophase (intermediate phase between solid and liquid, e.g., liquid-crystalline state). However, Yao et al. (2022) considered the event to be a reversible solid-solid phase transition of Form I to a new form named Form I; indeed, the material converted back to the initial state when cooled down (Fig. 3b).

X-ray diffraction patterns of Form I at variable temperature were collected to identify the nature of the phase transition (Fig. 4). Form I

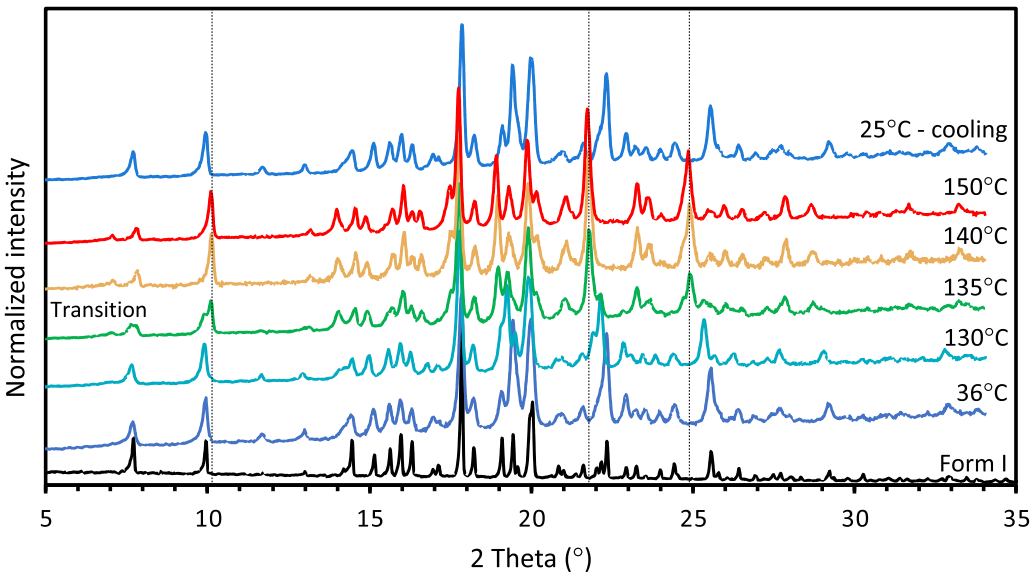

Fig. 4. Overlay of variable-temperature PXRD of Form I employing a heating rate of 10 °C/min. From the bottom: diffractogram of the reference Form I and Form I at 36, 130, 135, 140, 150 °C and after cooling the system back to 25 °C. The patterns at 140 °C and 150 °C correspond to Form I′. The diffractograms have been shifted on the vertical axis for a better representation.

exists as a single phase up to 130 °C; there is only a small shift of the reflections to lower values of 2θ due to the thermal expansion of the crystalline unit cell. The phase transition occurs near 135 °C (ΔH_t= 4.8 kJ/mol), as demonstrated by the presence of two sets of reflections belonging to two different crystal forms: Form I and a new form; the new form was named Form I′ as suggested by Yao et al. (2022). The new diffraction pattern of Form I′, with characteristic reflections at 10.1°, 21.7° and 24.9° 2θ, appears while the peaks of Form I (7.7° and 9.9° 2θ) are still present. When Form I′ is cooled down, it converts back to Form I as shown by the diffraction pattern collected at 25 °C after cooling. This spontaneous reconversion prevents a thorough characterization of Form I′ although it must be anhydrous as proven by DSC. Based on this data, the phase transition at 134.5 °C is an enantiotropic polymorphic transition (Bernstein, 2002) with Form I′ being the more stable of the two polymorphs at high temperature. Eventually, Form I′ melts at 167.9 °C (ΔH_m= 49.8 kJ/mol), as shown in the DSC (Fig. 3c).

The thermal behavior of a single crystal of Form I was also investigated by hot-stage polarized light microscopy and Raman microscopy at several temperatures. No macroscopic change in crystal morphology was observed during the phase conversion (supplementary material, Figure S43 and S44). The Raman spectra of Form I and Form I′ are almost superimposable, except for the shift of the peak at 745 cm^{-1} and change in relative intensity of the peaks at 1382 and 1402 cm^{-1}. The strong similarity of the Raman spectra suggests that the two crystal lattices are structurally closely related in terms of local environments and conformations of the posaconazole molecules.

The water-absorption behavior of Form I was tested by Dynamic Vapor Sorption (Fig. 5a). Form I adsorbs almost no water (0.2%) even when exposed to very high relative humidity; no form conversion was detected by powder X-ray diffraction (PXRD) at the end of the dynamic vapor sorption (DVS) cycles.

3.1.2. Form II

This form was originally called Form Y, however, to keep the nomenclature consistent, the notation Form II is preferred, as suggested by Yao et al. (2022) who recently solved its crystal structure. Form II is an anhydrous crystal form (no solvent loss in DSC and TG-FTIR), which melts at around 170 °C (supplementary material, Figure S7). Even if Form II is characterized by a high melting point, its melting enthalpy is lower than the one of Form I′ (Yao et al., 2022). Thermodynamic data suggest that Form II might be enantiotropically related to Form I and Form I'. Form II can be prepared by heating amorphous posaconazole (Din et al., 2020), Form III (Wieser et al., 2013) or other posaconazole channel hydrates above 115 °C. Form II is easily obtained from the amorphous material because the growth of nuclei is favored by the orientation of posaconazole molecule on the surface of the melt (Yao et al., 2022).

As in the case of Form I, the small amount of water uptake (0.4 % w/w) observed in the DVS of Form II is due to the adsorption of water molecules on the surface of the crystals (Fig. 5b).

3.2. Description of hydrate forms

3.2.1. Form III

Form III belongs to the class of non-stoichiometric solvates because the crystalline compound can exist with different numbers of solvent molecules inside the crystal lattice. Indeed, Form III has a channel-like structure that is able to accommodate molecules of water or methanol. Since no change in the crystal structure is observed after substitution of solvent molecules in the channels, we refer to the crystal form containing methanol molecules in the channels as Form III_m (m= methanol) and to the one containing water molecules as Form III_w (w= water). The presence of methanol molecules in voids was demonstrated by McQuiston et al. (2019) by solving the crystal structure. Since this form has only been obtained from experiments involving methanol (suspension of Form I or recrystallization), the molecules of solvent may act as a template for the formation of channels or, more in general, of the entire crystal lattice.

DVS of Form III_m using methanol vapor was performed in order to study the uptake of solvent molecules (Fig. 5d). This non-stoichiometric solvate, rapidly takes up methanol at low partial pressure and no hysteresis between sorption and desorption is observed. The channels, present in the crystal structure, quickly take up molecules of methanol up to 2.2 % w/w (1 molecule of methanol for every 2 molecules of posaconazole) and then, as the surface sites fill, the adsorption rate drastically decreases.

When Form III_m is exposed to air, water molecules substitute the methanol molecules contained in the channels because water, and not methanol, is present in the atmosphere. Only the release of water was detected in a TG-FTIR analysis performed on a sample of Form III_m

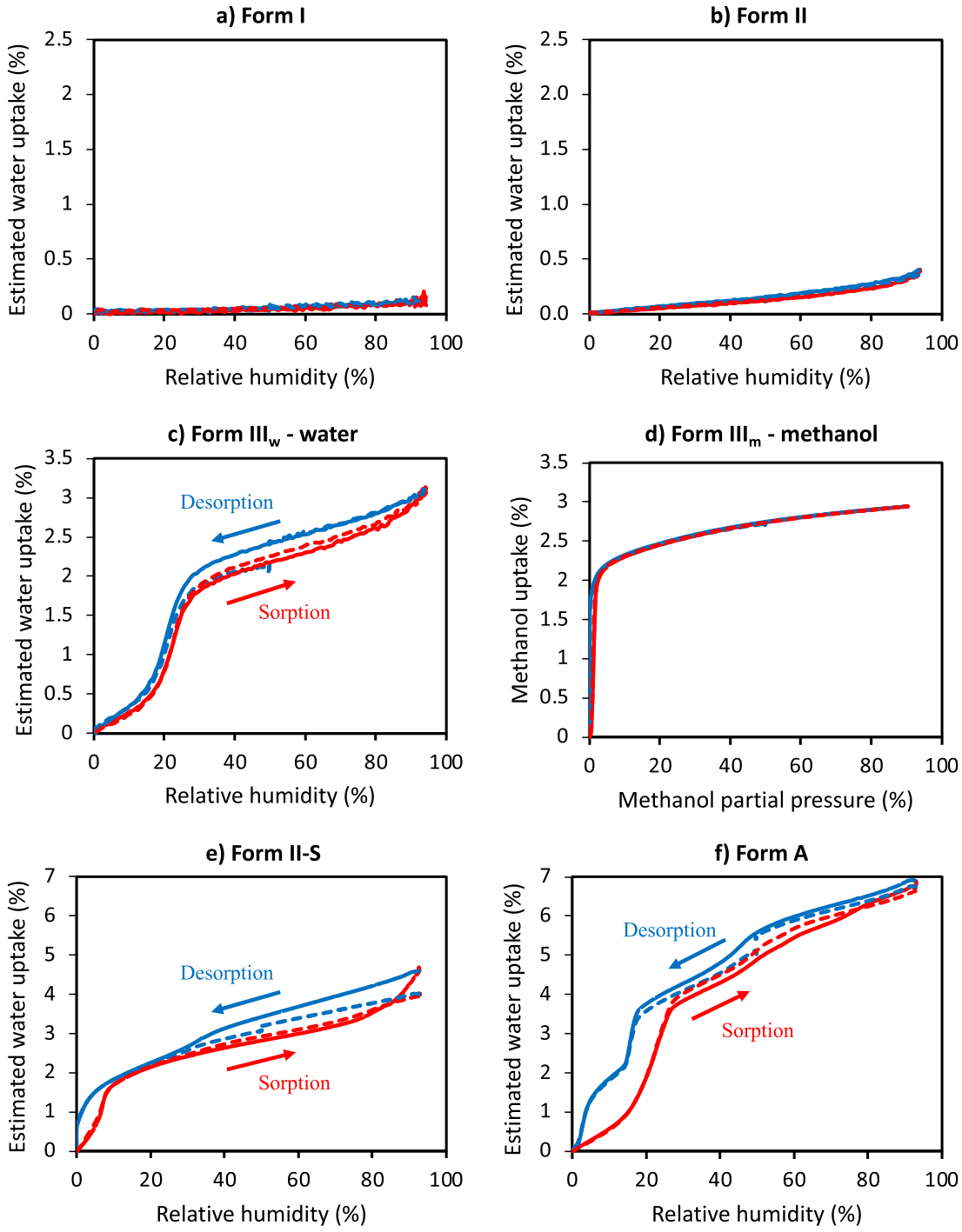

Fig. 5. Water sorption isotherms collected from 0% to 95% relative humidity of a) Form I, b) Form II, c) Form III$_w$, e) Form II-S, f) Form A. d) Methanol sorption isotherm of Form III$_m$ using methanol vapor from 0% to 90% partial pressure. Sorption cycles are depicted in red, desorption cycles in blue. The solid lines represent the first complete sorption-desorption cycle, and the dashed lines correspond to the initial desorption and the final sorption-desorption cycle. The water and methanol uptakes are expressed in mass percentage. Note that different scales are used in the various parts of the figure.

exposed to ambient conditions for 1 day (supplementary material, Figure S10 and S11). This hypothesis is further supported by the DVS measurement performed using water (Fig. 5c); in contrast to the anhydrous forms, Form III$_w$ absorbs 2.5 % w/w of water at 80 % relative humidity (i.e. approximately 1 mole of water per mole of posaconazole). The water sorption isotherm is characteristic of a non-stoichiometric hydrate because it shows a reversible release and uptake of water molecules, and almost no hysteresis between sorption and desorption (Coquerel, 2018). Therefore, Form III can be defined as a non-stoichiometric hydrate or non-stoichiometric solvate containing channels in the crystal lattice. The term Form III is used in this work to generally represent only one crystal form because both Form III$_m$ and Form III$_w$ are characterized by the same crystal structure with the possibility for the channels to be filled with methanol or water molecules, respectively.

The thermal behavior of Form III$_w$ was investigated by DSC and variable-temperature PXRD (Fig. 6). Form III$_w$ undergoes partial dehydration through a large temperature interval from 35 °C to 75 °C;

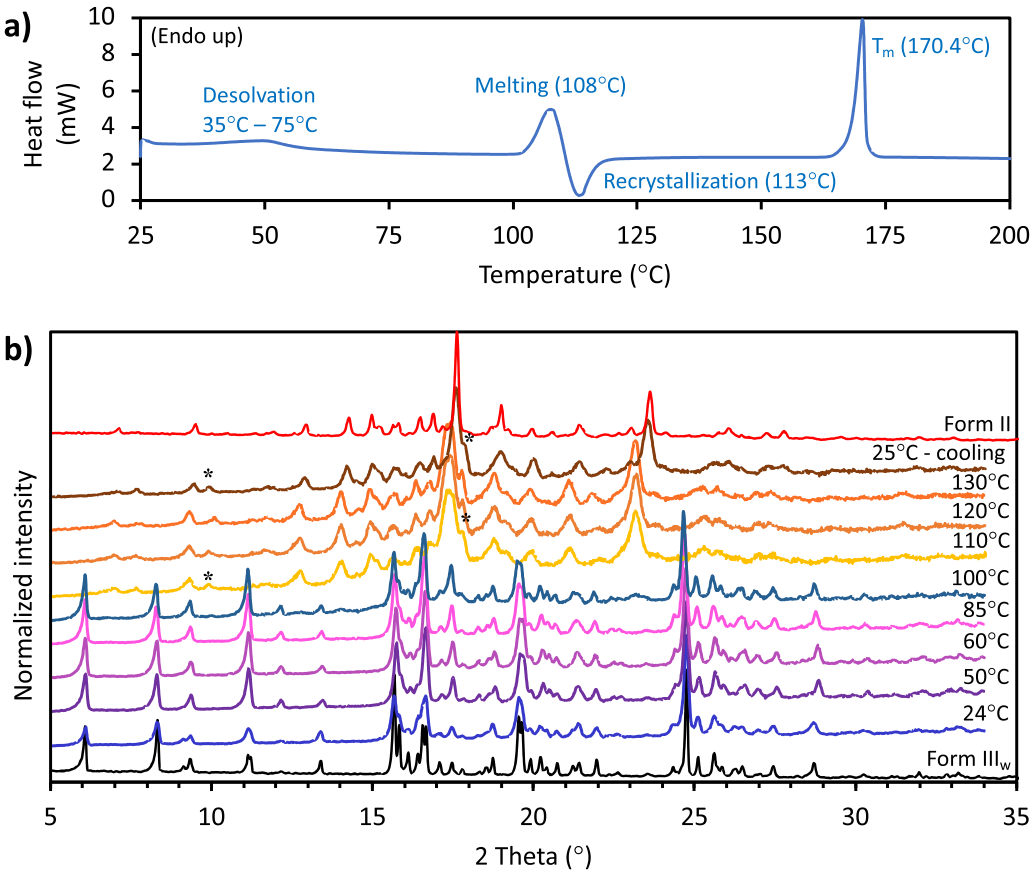

Fig. 6. a) DSC of Form III_w performed with a heating rate of 10 °C/min; b) Overlay of variable-temperature PXRD of Form III_w employing a heating rate of 10 °C/min. From the bottom: reference diffraction pattern of Form III_w, Form III_w collected at 24, 50, 60, 85, 100, 110, 120, 130 °C and after cooling the system back to 25 °C. The reference diffractogram for Form II is also depicted. The diffractograms have been shifted along the vertical axis for a better representation. (*) peaks of Form I (10.1° and 17.9° 2θ).

however, since molecules of solvent are contained in channels and not in the crystal lattice, no change in the solid structure is observed at these temperatures and a dehydrated hydrate is obtained. The compound starts melting at 105 °C but immediately recrystallizes into Form II, as shown by the diffraction patterns collected at 110 °C through 130 °C and after cooling down to 25 °C. The final melting point (170.4 °C) observed in the DSC corresponds to Form II. A few peaks of Form I can be observed in the final diffraction pattern: the crystal form may be produced during the recrystallization process. It is worthy to note that the melting point of the dehydrated Form III (approximately 108 °C) is much lower than those of Form I′ and Form II (167.9 °C and 170 °C, respectively). The thermal behavior of Form III_w explains why the channel hydrate and the channel solvate can be used in the preparation of Form II.

3.2.2. *Form II-S*

Wieser et al. (2012) synthesized Form II-S by adding water to a solution of posaconazole in acetone at reflux temperature and by cooling the obtained blend. However, an easier way to produce Form II-S has been found in the present work by conversion from Form I or amorphous posaconazole: the solid form is suspended in a water-acetone 50 % v/v blend for five days and then filtered.

Form II-S is characterized by an intense low angle reflection at 2.6° 2θ, indicating the presence of at least one long axis in the unit cell, and by peaks at 7.1°, 9.5° and 11.3° 2θ (Figure S13). The DVS of Form II-S suggests a non-stoichiometric hydrated nature of the crystal form: a gradual and reversible water uptake is observed during sorption and desorption (Fig. 5e). A total water uptake of 5 % w/w is obtained at 95 % r.h. (2 mol of water per mole of posaconazole). The small hysteresis at low relative humidity (below 10 % r.h.) suggests that some water molecules may be bound to specific locations in the crystal lattice. However, no change in the crystal structure is observed after DVS measurement or after drying under vacuum the sample (supplementary material, Figure S47).

The DSC of Form II-S is similar to the one of Form III_w: the solid loses water over a wide temperature interval (60–80 °C), melts near 110 °C and recrystallizes at 115 °C to Form II, which eventually melts at 170 °C (supplementary material, Figure S17). As in the case of Form III, the melting point of the dehydrated Form II-S (approximately 110 °C) is much lower than those of the two anhydrous forms.

3.2.3. *Form A*

Form A, which was named by Badone et al. (2015), is the third partially non-stoichiometric hydrate of posaconazole. Form A was previously obtained by antisolvent precipitation: water was slowly added as an antisolvent to a solution of posaconazole in acetone. A similar procedure was used in the patent of Andrews et al. (1999) to produce the so-called Form II.[1] However, the latter is not a new form but simply a poorly crystalline sample of Form A, as demonstrated by a diffraction pattern comparison (Table 1 and supplementary material, Figure S48). Both forms show the characteristic reflections at 4.3°, 6.3°, 9.3°, 12.6° and in the range 15–16° 2θ.

Antisolvent addition is a kinetic crystallization process involving a

[1] The Form II reported by Andrews et al. (1999) is not the same as the Form II reported by Yao et al. (2022); see Table 1. The Form II described by Yao et al. (2022) is an anhydrous form that coincides with the Form Y reported in the patent of Wieser et al. (2013).

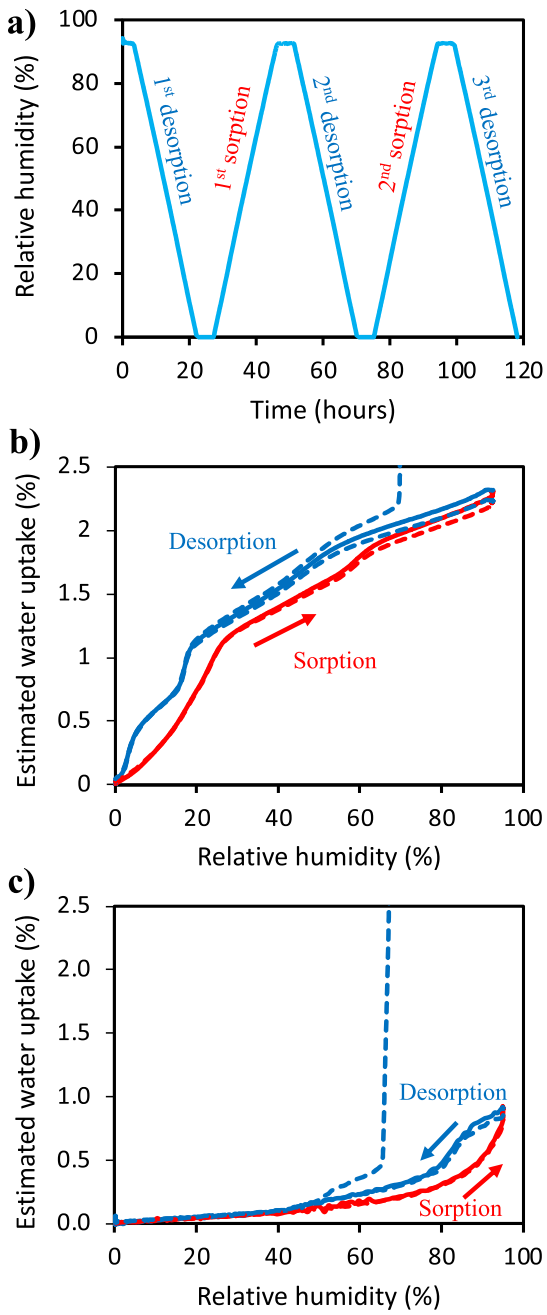

Fig. 7. DVS method (a) and water sorption isotherms collected from 0% to 95% relative humidity of two samples of Form S: b) sample tested after suspending Form I in water for 5 days; c) sample obtained after suspending Form I in water for 2 hours. Sorption cycles are depicted in red, desorption cycles in blue. The solid lines represent the first complete sorption-desorption cycle. The water uptake is expressed in mass percentage.

fast precipitation of the API and might result in a poorly crystalline phase. We found that suspending Form I in a THF and water mixture 50 % v/v for two days is a thermodynamically controlled procedure for obtaining Form A in a reproducible way.

The DVS of Form A shows the non-stoichiometric nature of the crystal structure; indeed, the reversible and continuous water uptake observed in the water sorption isotherm may be associated to the presence of channels (Fig. 5f). The weak interactions between water and posaconazole molecules is also supported by the release of water at low temperature (below 90 °C) observed in the TG-FTIR (supplementary material, Figure S20). However, the hysteresis below 30 % r.h. suggests that some water molecules may be bound to specific locations in the crystal lattice. A small change in the reflections at angles higher than 16° 2θ was observed after drying the sample (supplementary material, Figure S49). The change could be due to partial change of the hydrate structure, however further experiments are required to confirm the hypothesis such as single-crystal X-ray diffraction. The maximum water uptake of 7 % w/w indicates that Form A is able to accommodate more water molecules than other posaconazole crystal forms (around 3 mol of water per mole of posaconazole at 90 % relative humidity).

The thermal behavior of Form A is similar to those of the other hydrates. The DSC thermogram shows dehydration from 40 °C to 80 °C, melting with subsequent recrystallization into Form II at 120 °C and, finally, melting of Form II at 170 °C (supplementary material, Figure S22).

3.2.4. Form S

Form S is the form of posaconazole that is present in the marketed oral suspension (Lykouras et al., 2020) and is obtained by suspending Form I in water (Lykouras et al., 2023). The conversion in water suggests that Form S could be a hydrated form of posaconazole. Nevertheless, the characterization of the solid phase and the resolution of the crystal structure are very tricky because Form S is an elusive form that converts back to Form I when it is exposed to ambient air during sample handling and dries out. A technical option to avoid the re-conversion into Form I is the use of a wet sample of Form S. The diffraction pattern of the Form S was collected in transmission by sandwiching the wet powder between two acetate foils: the main reflections were observed at 7.2°, 10.2°, 14.6° and 17.8° 2θ (supplementary material, Figure S23) and agrees with those reported by Lykouras et al. (2020). The FT-Raman spectrum of Form S was collected by measuring a suspension of the sample contained in a NMR tube as described in Section 2.4.2. The same spectrum was collected by Raman microscopy focusing on solid particles contained in a suspension of Form S placed on a metal slide (supplementary material, Figure S24). It is worth noting that the Raman spectrum of Form S is almost completely superimposable to the one of Form I: the two forms share the same spectral features as represented by the peak at 740 cm^{-1} (Figure S45). This observation, in addition to the similarities observed in the diffraction patterns (Figure S46), suggests that the two crystal lattices are highly similar.

The hydrated nature of Form S could be confirmed by thermal analysis. However, performing a quantitative analysis of the wet powder by DSC or TGA is not possible because the residual water present on the surface of the particles would give misleading results.

For this reason, we performed a DVS analysis to identify the nature of the crystal Form S. The employed method is similar to the one reported in Section 2.4.5 but starts from 95 % r.h. (Fig. 7a). The sample of Form S was isolated after suspending Form I in water for 5 days. The solid form of the sample was confirmed by Raman microscopy before performing DVS measurements. The DVS started from 95 % relative humidity to gradually dry out the sample until reaching 0 % relative humidity; no pre-equilibration of the wet samples was performed at this stage. Surprisingly, the DVS profile of the sample (Fig. 7b) coincides with the water sorption isotherm observed for Form A but with a lower total water uptake. The profile would suggest partial conversion of Form S to Form A during the 5 days-long suspension in water (as further discussed in Section 3.5.2). In addition, no difference was observed between first and second desorption cycle meaning that the remaining Form S converted to Form I during the DVS measurement.

In a second attempt, Form S was isolated after suspending Form I in water for only two hours instead of 5 days to avoid conversion to Form A (Fig. 7c). The DVS profile shows a 0.9 % water uptake at 95 % relative humidity probably related to contamination of amorphous posaconazole present in the starting Form I. Also in this case, Form S completely converted to Form I during the first desorption cycle. However, the small step of water uptake observed at 60 % relative humidity of the first desorption cycle (dotted line Fig. 7c) could belong to Form S.

The DVS results confirmed the elusive nature of Form S and further studies are required to verify the hydrated nature of the form: a definitive proof of the nature of Form S would only be obtained by solving its crystal structure.

3.3. Description of solvate forms

Three solvates of posaconazole have previously been reported: methanol (Form III_m), isopropanol (Satyanarayana et al., 2013) and dioxane-water (McQuiston et al., 2019). As already discussed above, Form III contains channels in the structure able to accommodate molecules of methanol, although they are readily displaced by water. However, both dioxane and water molecules occupy lattice sites in the crystal structure of the dioxane-water solvate.

During this study, new solvates were discovered with N,N-dimethylformamide (DMF), pyridine and dichloromethane (DCM). All solvates were obtained by slow evaporation except for the DCM solvate, which was isolated by rotary evaporation, indicating that fast evaporation may lead to new kinetic solvates.

The solvates of pyridine, DCM and DMF appear to be nearly isostructural because of their similar diffraction patterns, with reflections at 3.1°, 6.2°, 7.6°, 9.3° and 14.6° 2θ observed for the three forms (supplementary material, Figure S50). The presence of solvent in the solid phase was confirmed by TG-FTIR analysis (supplementary material, Figure S33, S37, S41). The DCM solvate loses solvent over a large range of temperatures (40–100 °C), while the pyridine and DMF solvates lose solvent in single, narrow steps with inflection points at 85 °C and 95 °C, respectively.

3.4. Description of additional forms

Despite numerous attempts, we were not able to reproduce posaconazole Forms IV and V (supplementary material). Form IV was reported to be a non-stoichiometric hydrate and was previously obtained by conversion of Form III_m or amorphous posaconazole in a water–methanol solution 80:20 v/v for 6 days (Wieser et al., 2010). We performed several suspension experiments with amorphous posaconazole in mixtures of water–methanol at different water activity values. Depending on the water activity value, different polymorphs can be obtained (Li et al., 2008). However, we only observed the formation of Form A at high values of water activity (above 0.7) and Form III at lower values (below 0.6) after 7 days of equilibration.

Form V was previously obtained by fast evaporation of a dichloromethane (DCM) solution in a rotary evaporator (Gharpure et al., 2011). Our attempts resulted in the isolation of the DCM posaconazole solvate or amorphous material. Form V was not obtained by subsequent drying of the DCM solvate.

In addition, other new solid forms of posaconazole might be obtained by following kinetic crystallization processes not explored in this work. The case of Form IV and V illustrates how difficult it can be to reproduce crystal forms of an API. It is not only a matter of finding the right experimental conditions but a robust protocol, because the presence of seeds, impurities and fluctuations in relative humidity may drive the crystallization into the direction of an unsought form.

3.5. Conversion between crystal forms

3.5.1. Conversion of Form I in the presence of water and organic solvent

Above, at least three channel hydrates of posaconazole have been described: Form III_w, II-S and A. The formation of hydrates is quite surprising based on posaconazole's strong aversion for water. However, water molecules do not necessarily interact directly with the drug in the crystal lattice but can be hosted in empty channels contained in the crystal structure. The channel-like structure of the three hydrates is suggested by the continuous water uptake observed in the DVS analysis (Fig. 5). In addition to these forms, Form S is also likely a hydrate even if further analyses are required to confirm the hypothesis.

Table 2
Summary table of posaconazole hydrate forms and Form S reporting the solvent mixture employed in the isolation of the form and the maximum water uptake expressed both in percentage w/w and in moles of water per mole of posaconazole (not available for Form S).

Crystal form	Preparation solvent	Max water uptake (% w/w)	Max water uptake (moles of water)
Form S	water	n.a.	n.a.
Form III_w	Methanol + exposure to air	3	1.5
Form II-S	water - acetone 50% v/v	5	2
Form A	water -THF 50% v/v	7	3

All four crystal forms (III, II-S, A and S) can be obtained by conversion of Form I (the most stable anhydrous form at ambient conditions) in aqueous solution. We already discussed that the conversion of Form I to Form I′ occurs through rearrangement of the molecules in the solid phase, a process known as solid-solid phase transition. However, the conversion of Form I to the different hydrates is driven by the presence of solvent and occurs through a solvent-mediated phase transformation (Cardew and Davey, 1985). The solvent allows the dissolution of the starting solid phase and the nucleation and growth of a more stable form in solution. The isolated hydrate form depends on the organic solvent employed in the conversion experiment (Table 2): Form S is produced by suspending Form I in water, Form A by using a water-acetone blend, Form II-S by using a water-THF blend and Form III_w by using methanol or water–methanol blends and subsequently exposing to air. While the organic solvent enables the dissolution of Form I, which is too poorly wettable to dissolve in water, the selection of the isolated crystal form is probably related to the water activity of the water-organic solvent blend employed in the experiment.

Competitive slurries of the four forms were prepared in water in order to determine the most thermodynamically stable hydrate in water at RT. A complete conversion of Form S and Form II-S to Form A was observed after leaving the hydrates in water for 10 days (supplementary material, Figure S51, S52). Form III_w also converted into a poorly crystalline Form A when suspended in water–methanol mixtures rich in water content. The most stable hydrate at water activity of one was found to be Form A, which, as one would expect, is able to accommodate the highest number of moles of water per mole of posaconazole in the channels (Table 2).

3.5.2. Conversion of Form I in water to Form S

The conversion of Form I to Form S seems to follow an alternative route to the one of the other hydrates because no organic solvent is involved in the process. To better understand the transformation, a suspension of Form I in water was investigated by Raman microscopy over 5 weeks (Fig. 8). The technique enables the determination of the solid phase directly in a few drops of suspension, avoiding the isolation of the powder and the possible reconversion to Form I. Indeed, the Raman microscope spectrum of Form S collected from a few drops of suspension on a metal slide coincides to the FT-Raman spectrum collected as described in Section 2.4.2 from the suspension in the NMR tube (supplementary material, Figure S24). Since Form I tends to aggregate when added to water, the system was sonicated for few seconds in order to obtain a better homogenized suspension.

Form I converted to Form S almost immediately when suspended in water, as demonstrated by the characteristic Raman peak at 740 cm^{-1} observed after 2 minutes (Fig. 8). The process may be driven by the presence of channels in the crystal structure of Form S: the channels reduce the total free energy of the system in water by accommodating water molecule in their cavities. However, the rapidity of the conversion suggests a fast reorganization of the crystal lattice rather than a solvent-

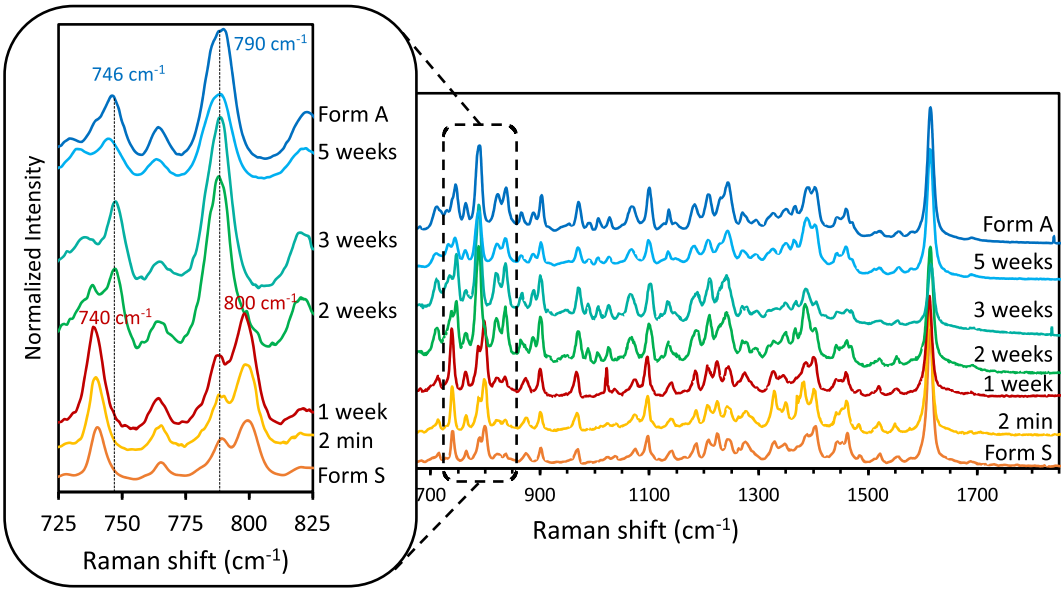

Fig. 8. Overlay of Raman microscopy spectra collected on a suspension of Form I in water over 5 weeks and magnification from 725 to 825 cm^{-1}. The spectra have been shifted along the vertical axis for a better representation.

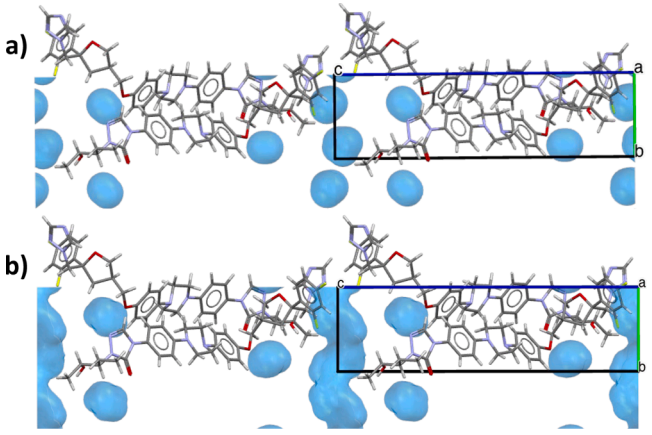

Fig. 9. Visualization of voids as light blue surface in the crystal structure of Form I (CCDC deposition number 1,828,463): a) use of a spherical probe with radius of 1.2 Å; b) use of a spherical probe with radius of 1.0 Å.

mediated phase transformation involving dissolution and reprecipitation. Such a fast reorganization could occur if channels are already present in Form I, but they are too narrow to pick up moisture. To confirm the hypothesis, the size and shape of empty spaces in Form I was analyzed using the "Hydrate analyzer" function available in Mercury software (version 2023.1.0., CCDC). The program displays the empty spaces of the crystal that are big enough to hold a spherical probe of a given radius (Macrae et al., 2020; Turner et al., 2011). The default probe radius is set to 1.2 Å, which is the approximate molecular radius of a water molecule (Barbour, 2006); a smaller value of the probe radius will identify smaller spaces and generate larger voids.

When using the default probe radius (light blue in Fig. 9a), the presence of isolated empty pockets is observed in Form I. However, when the probe radius is reduced (1.0 Å), the pockets connect together to form channels running in parallel to the c cell axis (light blue Fig. 9b). These channels are slightly too narrow to accommodate water molecules and for this reason no water uptake is observed in the DVS of Form I (Fig. 5a). However, exposing Form I to high values of relative humidity for a prolonged time may result in the conversion to Form S. In water, the process and its kinetics are different: direct exposure to solvent molecules may favor structure rearrangement and widening of the channels, allowing the accommodation of water molecules and reducing the free energy of the solid.

The presence of channels of Form S has not been demonstrated yet, and a definitive proof would be achieved only by the resolution of the crystal structure.

Even if Form S is characterized by lower free energy in water than Form I, it eventually converts to Form A, a thermodynamically more stable hydrate in water. Raman spectroscopy showed that the conversion starts after two weeks: the Raman spectrum shows the characteristic peaks of Form A at 746 and 790 cm^{-1} while the small peak at 740 cm^{-1} and the shoulder at 800 cm^{-1} can still be associated to the residual Form S (Fig. 8). However, DVS measurement performed on Form S (Fig. 7b) suggests that the conversion already starts after 5 days.

After three weeks, the features of Form S are not present anymore and the transformation to Form A can be considered completed. The conversion to Form A was confirmed by PXRD analysis of the isolated powder (Fig. 11b). The conversion takes longer in water than in THF – water blend because the dissolution of posaconazole is much slower in the absence of organic solvent. Since Form A is the most stable hydrate in water known to date, it is unsurprising that no further change in the Raman spectrum was observed after 3 weeks.

3.5.3. *Significance for the marketed oral suspension*

In the previous section, we showed that Form S completely converts to Form A in water within three weeks of storage. Indeed, Form A proved to be the most stable hydrate at a water activity of one in competitive slurry experiments. Surprisingly, the marketed aqueous oral suspension of posaconazole does not contain any Form A during the entire shelf life. Form S was exclusively detected by PXRD on a wet powder sample isolated from marketed oral suspension that had been stored for more than two years (supplementary material, Figure S23).

The persistent presence of Form S in the commercial suspension was suspected to be due to kinetic (or thermodynamic) stabilization provided by excipients contained in the formulation. Among the inactive ingredients, polysorbate 80 (or Tween 80), a nonionic surfactant, seemed to be the best candidate for interaction with posaconazole crystals. In the oral suspension the excipient is probably used in order to improve the wettability of the Form I particles. Indeed, a finely dispersed suspension of posaconazole raw crystalline material in water can be

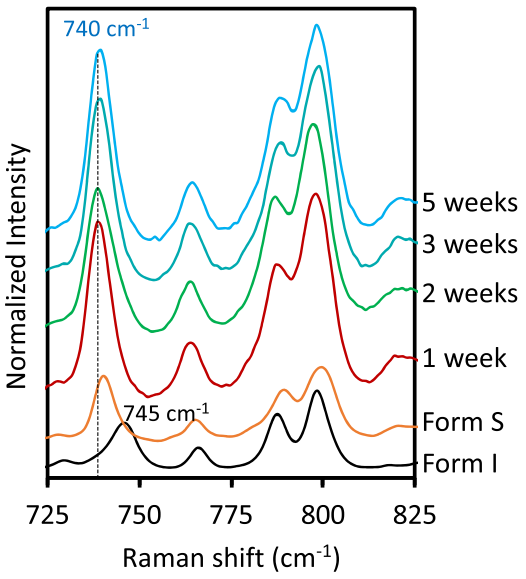

Fig. 10. Detailed region from 725 to 825 cm^{-1} of an overlay of Raman microscopy spectra collected on a suspension of Form I in polysorbate 80 aqueous solution (0.25% w/w) over 5 weeks. The spectra have been shifted along the vertical axis for a better representation.

achieved when polysorbate 80 is used.

To investigate the effect of the excipient on the hydrate stability, suspensions of Form I in polysorbate 80 aqueous solutions were monitored by Raman microscopy over a period of 5 weeks. Since the concentration of polysorbate 80 in the commercial formulation is unknown, two solutions of polysorbate 80 (0.01 % and 0.25 % w/w, respectively) were tested. Conversion of Form S to Form A was observed after 3 weeks with the lower concentration of the excipient; the PXRD of the isolated powder is reported in Fig. 11b. However, Form S was still present after 3 weeks in the 0.25 % w/w polysorbate 80 aqueous suspension, as demonstrated by the peak at 740 cm^{-1} observed in the Raman spectrum (Fig. 10) and by the PXRD of the isolated powder (Fig. 11b).

Based on the experimental findings, the kinetic stabilization of Form S in the posaconazole marketed oral suspension is related to the presence of polysorbate 80. The surfactant may affect the nucleation and crystal growth of the more stable Form A. A previous study reported that, in the presence of polysorbate 80 and hydroxypropyl methylcellulose (HPMC) in aqueous solution, the conversion of anhydrous piroxicam to the hydrate form was prevented (Kirchmeyer et al., 2015). The ability of these surfactants to stabilize crystal forms by interacting with the surface of the particles is well known. Indeed, surfactant and polymer adsorption on the surface of already formed crystals can reduce the crystal growth rate by several orders of magnitudes (Lindfors et al., 2008). For the conversion of Form S to Form A, where the conversion seems to occur through dissolution of the pre-existing hydrate and recrystallization of the most stable form in bulk solution, the effect may be similar. In this case, polysorbate 80 may preferentially mask sites on crystal surfaces that are suitable for nucleation. For this reason, further experiments are required in order to understand the mechanism of stabilization in more detail.

4. Conclusion

Posaconazole is a weakly dibasic drug characterized by a rich solid form landscape; a plethora of polymorphs and hydrates of the compound were previously discovered but little was known about their nature and their stability relationships. In this paper, a description of anhydrous, hydrated and solvated forms of posaconazole is reported. The phase conversions of polymorphs and hydrates were investigated by thermal analysis and by suspension in water to assess the relative stability of the crystal forms. The collected data were used to interpret the solid-phase transformations in water of posaconazole Form S, the crystal form contained in the marketed oral suspension. Form S was demonstrated to convert to the thermodynamically more stable hydrate, Form A, after suspending the system in water for 2 weeks. However, Form S is kinetically stabilized in the marketed oral suspension by the presence of polysorbate 80. Since the polymer may prevent nucleation or inhibit crystal growth of Form A, further experiments are required to elucidate the effect of the polymer on the stabilization mechanism. The information about posaconazole crystal forms contained in this paper might be useful not only for drug product development but also for precipitation studies of supersaturated posaconazole systems and the prediction of

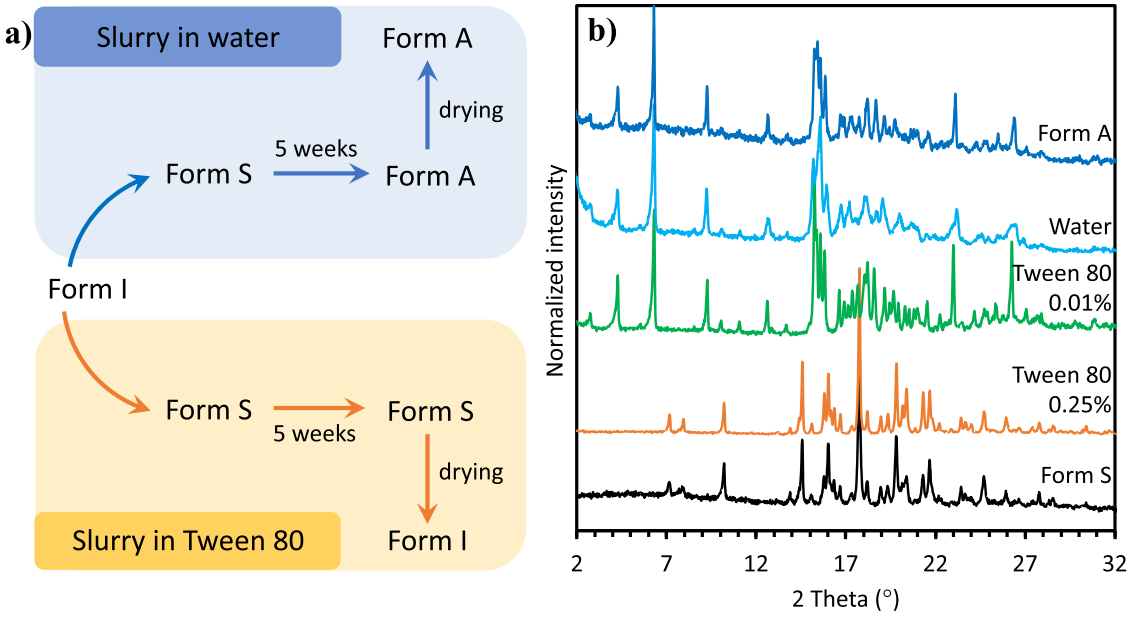

Fig. 11. a) Schematic representation of the conversion of Form I in water and in a polysorbate 80 (Tween 80) aqueous suspension (0.25% w/w); b) Overlay of diffraction patterns; from the bottom: Form S, isolated powder obtained after 3 weeks in polysorbate 80 aqueous suspension (0.25% w/w), isolated powder obtained after 3 weeks in polysorbate 80 aqueous suspension (0.01% w/w), isolated powder obtained after 3 weeks in water, Form A.

product performance.

CRediT authorship contribution statement

Matteo Guidetti: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing, Data curation. **Rolf Hilfiker:** Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. **Martin Kuentz:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision. **Annette Bauer-Brandl:** Conceptualization, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Fritz Blatter:** Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing, Project administration.

Declaration of competing interest

None

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2024.106722.

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