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Research Paper

Effects of Moisture and Residual Solvent on the Phase Stability of Orthorhombic Paracetamol

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Purpose. At high relative humidity (RH), orthorhombic paracetamol (form II) crystallized from ethanol transforms to monoclinic (form I) faster than such crystallized from the melt. The present study attempts to elucidate the reasons for this difference in stability.
Methods. The transformation of form II was investigated by powder X-ray diffraction, optical microscopy, gravimetric moisture sorption, thermogravimetry, and vibrational spectroscopy.
Results. Solution-grown form II was found to be always contaminated with form I nuclei but still transforms much faster than corresponding physical mixtures of the pure forms in high RH, at a rate that is depending on the RH and the size of the crystals. A 0.1–0.6% w/w mass loss, inversely related to the initial monoclinic content, was observed during transformation of solution-grown form II, and was found to be due to residual ethanol that could not be removed by grinding, indicating incorporation by a solid solution mechanism.
Conclusions. Moisture triggers the growth of existing form I nuclei but it exerts a weaker effect on nucleation, and the presence of residual ethanol greatly accelerates the transformation.

KEY WORDS: crystal polymorphism; moisture-induced phase transition; orthorhombic paracetamol; residual solvent; solvent inclusion.

INTRODUCTION

Paracetamol exists in three crystal forms, form I, which is stable at room temperature and belongs to the monoclinic system (1), form II identified as orthorhombic (2), and form III, which is very unstable and has not been fully characterized (3). Additionally, several molecular adducts have been identified (4) including solvates and hydrates (5,6). The orthorhombic polymorph is metastable at ambient conditions (7), however, the thermodynamic relationship with the monoclinic form is obviously a quite controversial issue. Some literature reports based on DSC data suggest an enantiotropic relation with an expected form I to II transition point below 263 K (8–10), while others based on adiabatic calorimetry (11), high pressure DSC (12) or sublimation and solution calorimetry (13), suggest a monotropic relation and rule out the possibility of a form I to II transformation at atmospheric pressure. Nevertheless, form II is of particular interest in tablet manufacturing, because it has well-defined slip planes in its crystal lattice and is suitable for direct compression (7). Several methods of crystallization of orthorhombic paracetamol from solution, some of them suitable for industrial use, have been reported in the literature, such as by seeding of ethanol solutions with melt-grown form II crystals (14), by the use of polymer heteronuclei (15), by high pressure crystallization (16), and by slow cooling of water solutions under firm control of nucleation conditions (17). However, the only reproducible method is the seeding technique (14). This process has been extensively studied (18–20) and it was found that harvesting and drying procedures are the most critical steps affecting polymorphic purity (14), because prolonged contact with the solvent induces transformation to form I (19). Regarding the long-term polymorphic stability of the orthorhombic crystals, conflicting data can be found in the literature, depending on the crystallization method (melt or solution) or even on the solvent used. Crystals grown from ethanol solutions, once properly dried, are stable when stored at dry atmosphere (14), while large orthorhombic single crystals produced from water solutions have been reported to transform to form I regardless of storage conditions, due to the existence of residual water inclusions in the crystal lattice (11). On the contrary, orthorhombic paracetamol crystallized from the melt has been reported to be stable over an 11 months period (7) without requiring any special precautions regarding the storage conditions.

In spite of the great industrial importance of the orthorhombic polymorph, difficulties in scaling up production in combination with the aforementioned controversy regarding the transformation to form I during long-term storage have hindered its use in commercial preparations. Although the seeded crystallization method has been optimized (20), and the solvent-mediated transformation has been investigated (19), until today there has been no systematic study on the

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mechanism of the solid-state transformation to the monoclinic form during storage. Thus, the present study attempts to elucidate the factors affecting stability of orthorhombic paracetamol, and particularly the effects of atmospheric relative humidity (RH) and residual solvent on crystalline samples of different initial monoclinic content, by applying powder X-ray diffraction (PXRD) in combination with gravimetric dynamic moisture sorption, thermo-analytical methods, vibrational spectroscopy and optical microscopy.

MATERIALS AND METHODS

Materials

Crystalline paracetamol, monoclinic form, Ph.Eur.-grade, obtained from APOKA, (Apoka Pharma Produktions und Handelsgesellschaft m.b.H., Vienna, Austria), was used for the preparation of orthorhombic seeds from melt and for the crystallization from ethanol solutions. Ethanol (96%, b.p. 79°C) was used as solvent.

KNO_3 of analytical grade (99% purity, Merck, Darmstadt, Germany) was used for the creation of 92% RH atmosphere in closed desiccators.

High-boiling point silicone oil (Wacker Chemie AG, Munich, Germany) was used as a suspension medium for thermo-microscopic observation.

Methods

Crystallization of Orthorhombic Paracetamol from the Melt

Pure orthorhombic paracetamol was produced from the melt by placing approximately 10 g of substance in a sealed glass ampoule and heating to 200°C for 10 min to ensure complete melting. Afterwards, the melt was allowed to cool down to 70°C, in order to induce the nucleation and growth of form II. The obtained crystalline mass was gently ground to a fine powder using a mortar and pestle, and afterwards stored in desiccators over phosphorous pentoxide. Polymorphic identity and purity were verified by PXRD and the crystals were used as seeds in the solution-growth experiments and as reference for highly pure form II.

Crystallization of Orthorhombic Paracetamol from Solution

This crystallization was conducted by cooling supersaturated ethanol solutions, as described previously (18). The apparatus consisted of a 250 ml jacketed wall glass crystallizer (Schmizo, Zofingen, Switzerland), connected through twin valves to both a refrigerated (Lauda Proline RP 855, Lauda-Königshofen, Germany) and a heated (Haake, Karlsruhe, Germany) circulator, to allow the rapid change of the circulating fluid. An amount of 50 g of crystalline paracetamol was dissolved in 150 ml of ethanol at a constant temperature of 50°C. After complete dissolution, the solution in the crystallizer-vessel was kept still applying no agitation and its clarity was checked. Immediately after clarification, the circulation of the warm (50°C) water was stopped and cooling was commenced by circulating anti-freeze fluid of −20°C. The solution was seeded after 10 min at a temperature approximately 0°C by adding ~5 mg of melt-grown form II crystals and agitation was applied at 350 rpm using an RZR 2021 stirrer (Heidolph Instruments GmbH, Schwabach, Germany) equipped with a glass paddle. The crystals were harvested 20 min after seeding by a quick vacuum filtration. The wet material was spread on a 100 μm sieve nested inside a glass funnel and covered with a filter paper in order to prevent loss of crystalline material while dry air was blown through the funnel outlet for about 15 min. The crystals were afterwards stored in desiccators at 0% RH (phosphorous pentoxide). This procedure guaranteed a minimal transformation to the stable monoclinic form during the harvesting process.

A total of seven batches of different polymorphic purity were produced, three highly pure in form II following the described harvesting and drying procedures, three of intermediate purity by prolonging the filtration time before drying with cold air, and one of very low form II content, that was left to dry in the atmosphere after filtration without application of an air stream.

PXRD Analysis and Monitoring of Moisture-induced Transformation

The crystal form of the samples was verified by powder X-ray diffraction, using a Siemens D-5000 diffractometer (Siemens AG, Karlsruhe, Germany) equipped with a theta/theta goniometer, a CuKα radiation source, a Goebel mirror (Bruker AXS, Karlsruhe, Germany), a 0.15° soller slit collimator, and a scintillation counter. The patterns were recorded at a tube voltage of 40 kV and a current of 35 mA applying a scan rate of 0.005° 2θ/s in the angular range of 2–40° 2θ.

Additionally, for monitoring of the moisture-induced transformation of highly pure solution-grown form II crystals, the same diffractometer, equipped with a low temperature chamber (TTK, Anton Paar, Graz, Austria) interfaced with a SETARAM-WETSYS humidity generator (KEP-technologies, Pennsauken NJ, USA), was used to control the relative humidity in the chamber at 85% and 90% RH at 25°C. The powder sample was scanned continuously until complete transformation in the range of 9–28° 2θ.

Optical Polarized Light and Hot-Stage Microscopy

Microscopic observations of the crystalline samples were performed on an Olympus BH-2 optical polarization microscope (Olympus Optical GmbH, Vienna, Austria) equipped with polarizer and analyzer. For the observation of the moisture-induced transformation, a VGI2000M accessory (Surface Measurement Systems Inc, London, UK) was attached to the microscope and the sample was conditioned at 95% RH and a temperature of 25°C. For thermomicroscopic investigations the microscope was equipped with a Kofler hot stage (Reichert Thermovar, Vienna, Austria). Photomicrographs were acquired using an Olympus DP50 microscope stage digital camera with a resolution of 5.8 million pixels, operated through the AnalySIS 3.2 software (Soft Imaging System GmbH, Hamburg, Germany).

FT-Raman Spectroscopy

FT-Raman spectra were recorded on a Bruker RFS 100 FT-Raman spectrometer, equipped with a diode pumped Nd:

YAG laser (1,064 nm) at an output power of 200 mW as the excitation source and a liquid nitrogen cooled, high sensitivity Ge detector (Bruker Optik GmbH, Ettlingen, Germany). The powder samples were packed into small aluminum cups and the spectra were recorded with 64 scans per spectrum over a range from 4,000 to 10 cm^{-1} at a resolution of 4 cm^{-1}. The monoclinic content of the crystals was determined applying a multivariate calibration method based on PLS regression, described in detail elsewhere (21). Additionally, the solvent-induced transformation of form II was monitored with the same FT-Raman spectrometer at 25°C, using a 5 mm test tube. After the tube was filled (about 15 mm high) with crystals of pure orthorhombic form grown from the melt, the sample was slightly compressed and ethanol was added. The tube with the suspension was immediately placed on the Raman sample holder and the recording of spectra was started.

Gravimetric Vapor Sorption/Desorption Analysis

Dynamic water vapour sorption and desorption was gravimetrically measured using an automatic multi-sample moisture sorption analyzer SPS11 (Projekt Messtechnik, Ulm, Germany) at 25°C. Approximately 1.0 g of accurately weighted crystals (balance resolution: 10 μg), intact and shattered by manual grinding using mortar and pestle were placed in an aluminium sample dish (diameter: 50 mm) and transferred in the moisture sorption analyzer equilibrated at 0% RH. The mass change of the material was recorded every 8 min with the equilibrium condition set to <0.001% within 35 min and the maximum residence time to 12 h for each RH level. When the equilibrium condition was fulfilled or the maximum residence time exceeded, the relative humidity was automatically increased by 10% steps up to 90% RH, then by 5% to 95% RH, and then decreased down to 0% RH in the same stepwise manner (interval method). The mass change at equilibrium condition (or maximum residence time) was used to draw the moisture sorption isotherms.

Furthermore, measurements were conducted at constant relative humidity of 85% and 90% at 25°C for the purest solution-grown batch of form II (batch 1), both intact and ground, until complete transformation of the sample.

Karl-Fisher Titrimetry

The residual water content of dry samples (equilibrated at 0% RH above phosphorous pentoxide) was measured by volumetric Karl-Fischer titration, using a DL38-titrator (Mettler-Toledo AG, Greifensee, Switzerland). Pyridin-free Apura Solvent and Apura Titrant5 (Merck, Darmstadt, Germany) were used for the titration. Amounts of 50–100 mg of the substance were accurately weighted (mass controlled to ±0.01 mg using an AT250-balance, Mettler, Greifensee, Switzerland) into a weighing boat and put into the titration vessel.

TGA Determination of Total Volatile Impurities

The absolute volatile impurity content of the samples, which were previously equilibrated above phosphorous pentoxide, was measured by TGA. Accurately weighted (±0.0005 mg) samples of approximately 10 mg were placed into 50 μl platinum pans and subjected to non-isothermal TGA at a heating rate of 10 K/min in a temperature range from 25°C to 200°C using a Perkin-Elmer TGA-7 system (Perkin-Elmer, Norwalk, CT, USA) operated through the Pyris 2.0 software. Nitrogen was used as purge gas (balance purge: 50 ml/min, sample purge: 25 ml/min). The percent weight change was calculated in the range of 70–135°C, where the transformation to form I occurs.

TG-FTIR Evolved Gas Analysis for the Identification of Volatile Chemical Species

The coupled TG/FTIR measurements were performed by heating at the same heating rate (10 K/min) under dry nitrogen flux of 50 ml/min in the range of 25°C to 200°C using a Netzsch TG 209 thermogravimetric analyzer (Netzsch Instruments Inc., Selb, Germany). The outlet tube of the thermobalance was connected to a FTIR gas cell, which was maintained at 200°C and placed inside a VECTOR22 FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany). Spectra were recorded over a range from 4,500 to 550 cm^{-1} at an instrument resolution of 4 cm^{-1} (20 scans per spectrum).

Moisture-Induced Transformation Kinetics

The mechanism of the gravimetrically monitored isothermal moisture-induced transformation of orthorhombic paracetamol was investigated by means of local Avrami exponents (22). The local value of the Avrami exponent at a certain transformed fraction $n_{(\alpha)}$ is calculated by the equation:

$$n_{(\alpha)} = \frac{\partial\{\ln[-\ln(1-\alpha)]\}}{\partial[\ln(t)]} \quad (1)$$

where α is the transformed fraction and t is the time. The $n_{(\alpha)}$ values, which are related to the reaction mechanism (23), are plotted *versus* the transformed fraction, α, and any changes in the mechanism throughout the transformation are easily identified from the changing slope of the curve. Similarly to model-free methods, this method does not assume a constant mechanism throughout the transformation, and offers the advantage of "tracking" the changing mechanism during the reaction.

RESULTS AND DISCUSSION

In Fig. 1 the PXRD patterns of the crystals obtained from ethanol solutions are presented with the reference patterns of the commercial monoclinic form I and melt-grown form II, while in Table I the monoclinic content is listed together with the corresponding drying conditions (approximate filtration time and drying method). All solution-grown batches contain varying amounts of form I, which shows a general dependence upon the applied filtration and drying procedures. Samples dried by a stream of cold air immediately after quick filtration have the highest purity (between 4% and 12% *w*/*w* of the monoclinic form I). Our observations indicate that the large variation in the content of form I of similarly crystallized samples is due to the fast transformation

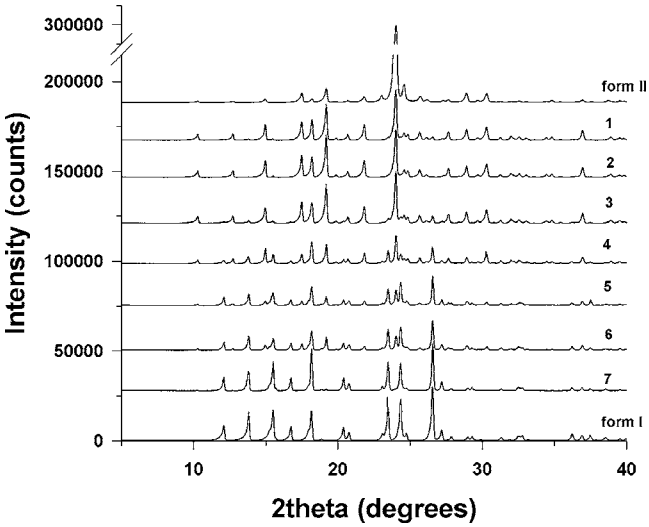

Fig. 1. PXRD patterns of the crystals obtained from ethanol solutions (batches 1–7) in comparison with those of the pure form II grown from the melt, and the commercial monoclinic form

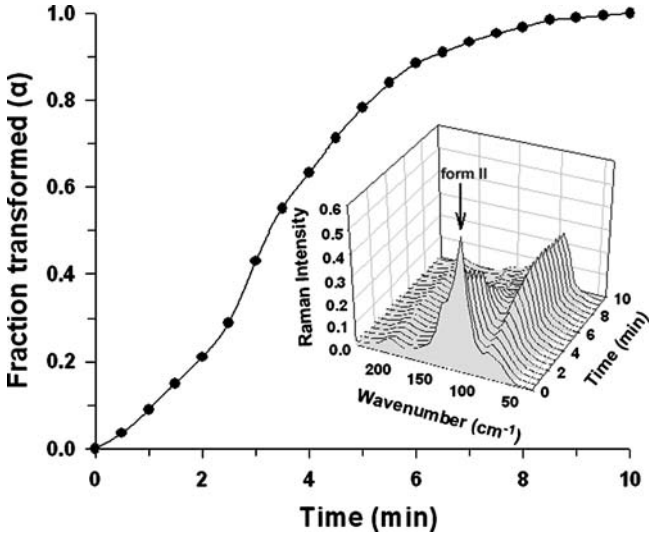

Fig. 2. Transformed fraction *versus* time, calculated from the intensity of the 122 cm^{-1} peak of sequentially recorded FT-Raman spectra of form II crystals suspended in ethanol

of form II in the presence of the solvent/mother liquor and not because of primary nucleation and growth from the solvent. This outlines the strong influence of minor variations in harvesting and drying of freshly crystallized form II samples on the phase purity of the final product. In order to verify this assumption, dry samples of pure form II crystals grown from the melt were placed in a test tube (5 mm in diameter) and ethanol was added at ambient temperature. The transition kinetics of the orthorhombic to the monoclinic form was monitored by sequentially recording FT-Raman spectra of this suspension. The transformed fraction of form II was calculated from the intensity of the 122 cm^{-1} peak, and plotted *versus* time. As shown in Fig. 2, the transformation takes place rapidly. The curve is nearly sigmoidal indicating a nucleation and growth mechanism. However the initial phase of the time curve, which is dominated by the nucleation process, is unusually steep and almost linear. This can be explained by the fast nucleation of form I at ambient temperature, which can grow right from the beginning. The maximal transformation rate occurs after about 3 min followed by a decelerating transition process towards the end of the reaction. Anyway, the high transformation rate supports our view that a solvent-induced transformation is the main reason why solution-grown form II crystals are contaminated with small amounts of form I. The possibility of primary nucleation of both forms in solution and prevalence of form II due to a higher nucleation rate cannot be ruled out, however it seems highly unlikely based on previously published data (19) and considering that in a seeded solution, primary nucleation is not expected to take place. In other words, we infer from our studies that the stable form I does not arise directly by a primary nucleation process in the highly supersaturated solution but is the product of a solvent mediated transformation process of the metastable orthorhombic form II.

Table I. Monoclinic Content and Corresponding Drying Condition Applied, for the Different Orthorhombic Paracetamol Batches Used in this Study

Batch Code number	Form I Content (% *w/w*)[a]	Drying Conditions (Filtration Time—Drying Method)
1	3.76	<5 min—cold air stream
2	8.30	<5 min—cold air stream
3	14.65	<5 min—cold air steam
4	29.75	≥5 min—cold air stream
5	56.49	≥5 min—cold air stream
6	62.75	≥5 min—cold air stream
7	98.53	≥5 min—drying in the open air
Form II (melt)	0.00	–
Form I	100.00	–

[a] Root mean squared error of prediction (RMSEP) <0.5%

The observation that a metastable form (kinetic form) crystallizes first and then transforms to a more stable polymorph is very common and coincides with Ostwald's rule of stages (24). In the case of paracetamol our observations are in good agreement with previous reports that stress the importance of the fast removal of the solvent (14). We may also note that though the applied crystallisation procedure gives "concomitant polymorphs" (25), the formation process of the two forms cannot be classified as "concomitant crystallization" but a consecutive or "seriatim crystallization" consisting of two steps, the crystallization of the metastable form II from solution and the subsequent transformation of this form to the stable form I.

The effect of water vapor and initial form I content on the transformation of pure form II was studied by storing pure (melt grown) form II crystals as well as physical mixtures of this sample with 5% *w/w* form I, at 92% RH in closed desiccators over saturated KNO_3 solutions for up to 5 weeks. The X-ray diffractograms of these samples (Fig. 3) show that melt-grown form II is resistant against the effect of moisture for more than a week, and even after a 5 weeks period, the transformation to the stable form was not complete. On the contrary, when seeded with form I (5% *w/w* mixture with form I), form II transformed to a large extent within a week. This is a very clear indication that the presence of form I nuclei in batches of form II greatly accelerates the transfor-

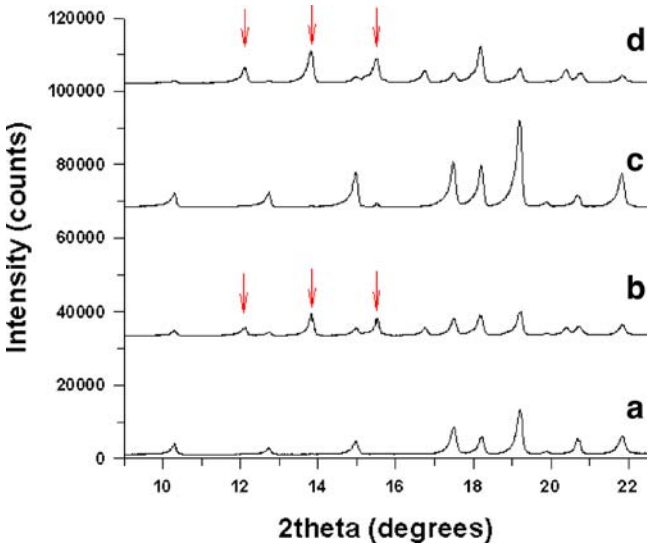

Fig. 3. PXRD patterns of paracetamol crystals grown from the melt after storage at 92% RH for one (*a*) and 5 weeks (*b*), of 5% *w*/*w* form I mixtures before (*c*) and after storage at 92% RH for 1 week (*d*). *Arrows* indicate peaks of form I

mation at high relative humidity that otherwise proceeds much slower.

The moisture-induced transformation of the orthorhombic form obtained from ethanol solutions as well as from the melt, was further investigated by gravimetric moisture sorption/desorption experiments. Corresponding isotherms are presented in Fig. 4. The pure form II crystals (melt grown) and commercial form I (Fig. 4a,b respectively), take up very little water even at 95% RH. Surprisingly, the crystals grown from ethanol solutions undergo a remarkable mass loss, which starts at about 60% RH and accelerates at higher RH until a complete transformation to form I, which was verified by PXRD. This means that the transformation can be monitored gravimetrically. The observed mass loss seems to be inversely related to the monoclinic content, and for the purest form II batch (batch 1) the total mass loss is slightly lower than 0.6% (Fig. 4a). It is also evident from the curve of the ground batch 1 (dotted line in Fig. 4a) that crystal size reduction exerts a negligible effect on the total mass loss, but significantly changes the kinetics of the phase transition. The mass loss of the ground material is higher than that of the intact sample at any condition below 95% RH. Since the data points are not in equilibrium in the 12 h residence time limit of each humidity step, the difference in the mass change curves of the ground and intact batch 1 sample is only an effect of different reaction rates.

In order to understand the mass loss during the moisture induced transformation and to identify the volatile impurity, TGA experiments, Karl-Fischer titrimetry and TG-FTIR were additionally applied. Table II lists the results of the mass loss of the different batches determined with gravimetric moisture sorption and TGA together with the water content of the crystals, determined by Karl-Fischer titrimetry. The TGA results show a slightly smaller mass loss than the moisture sorption experiments. The volatile impurity could be removed only by prolonged thermal annealing of the samples (140°C for 24 h) that always results in the transformation to form I, but not by grinding of the crystals prior to the TGA run. This annealing procedure always involves the transformation to form I. Since the water content of the crystals is very small it becomes obvious that the mass loss cannot be attributed to surface water desorption or release from inclusions in the crystal during the transformation to form I. TG-FTIR analysis, Fig. 5, finally proved that the volatile impurity is ethanol. Most of the solvent is released during the transformation process to the monoclinic form, and only a very small amount remains until melting (169–171°C). This indicates that the solvent can be incorporated in the crystal

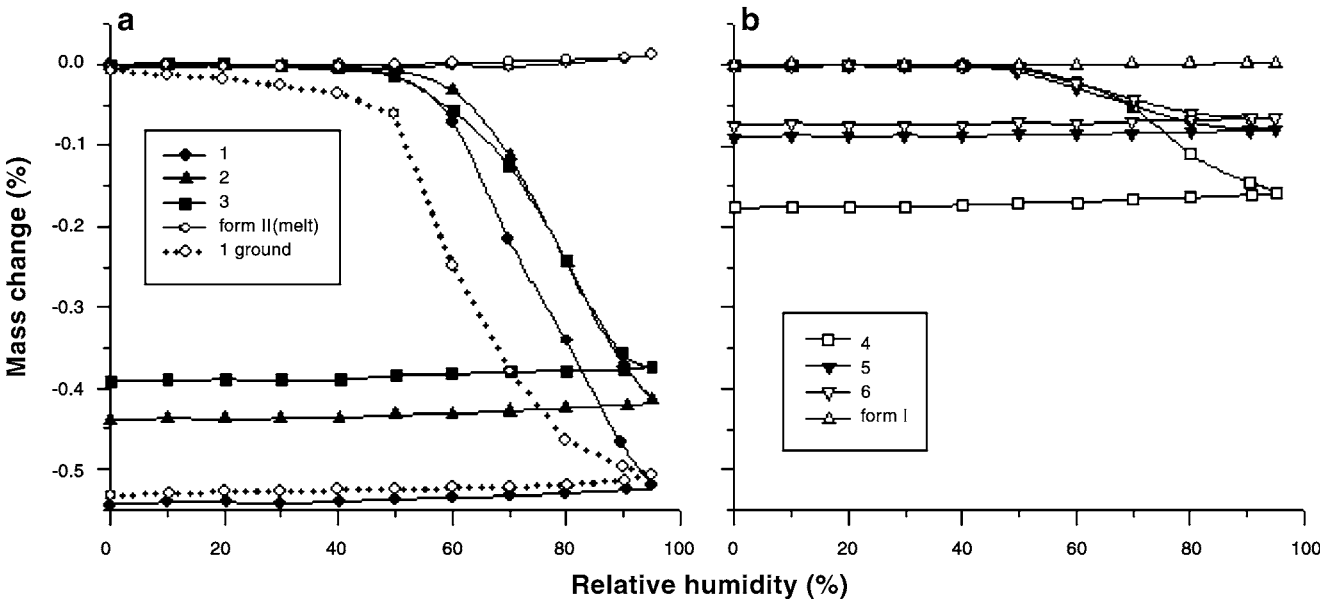

Fig. 4. **a**–**b** Moisture sorption isotherms of the orthorhombic paracetamol samples obtained from ethanol solutions (graph **a**: ground and intact batch 1, 2 and 3; graph **b**: batch 4 to 6), from the melt (graph **a**) and commercial monoclinic form (graph **b**). The mass change of the sorption cycle from 50 to 95% RH is not at equilibrium

Table II. Mass Loss Measured by Gravimetric Moisture Sorption and TGA, and Corresponding Water Content Determined by Karl-Fisher Titration, for the Different Orthorhombic Paracetamol Batches Crystallized from Ethanol Solution

Batch Code Number	Mass Loss Determined by		Water Content by Karl-Fischer Titrimetry (% *w/w*)[c]
	Moisture sorption (% *w/w*)[a]	TGA (% *w/w*)[b]	
1	0.53	0.40	0.05
2	0.42	0.31	0.04
3	0.41	0.30	0.02
4	0.18	0.13	0.01
5	0.08	0.07	0.01
6	0.07	0.05	0.01
7	0.00	0.00	0.01
Form II (melt)	0.00	Decomp.	0.00
Form I	0.00	0.00	0.02

[a] SD<0.05
[b] SD<0.03
[c] SD<0.02

structure of the orthorhombic form but hardly in the structure of monoclinic form. This fact is also evident from plots of the percent mass loss or ethanol content *versus* the monoclinic content on a logarithmic scale, Fig. 6, which gives a log–linear relationship between the amount of residual ethanol and the monoclinic content of the crystals. Further arguments that the mechanism of incorporation must be different to physical liquid inclusion, are due to the microscopic observation that revealed no visible pockets and the observation that the ethanol content cannot be reduced by grinding. Therefore, it seems most probable that the solvent is incorporated in the crystal structure of form II at the molecular level, following a solid solution mechanism, as described by Zhang and Grant (26,27).

The transformation kinetics (Fig. 7) of the different form II samples were gravimetrically monitored at constant humidity (85% and 90% RH) and temperature (25°C). Additionally, untreated (intact) and ground crystals of batch 1 were recorded to roughly evaluate the impact of crystal size (or surface area) on the transformation behaviour. The mass loss and hence transformation to form I is complete in less than 2 days, indicating that the presence of residual ethanol in

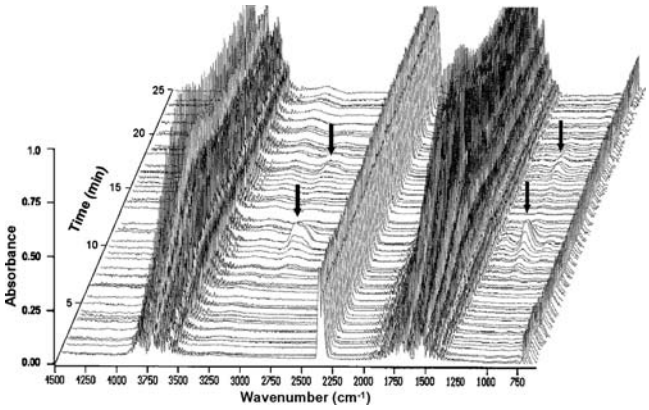

Fig. 5. Sequentially recorded FTIR spectra of the evolved gases during heating of orthorhombic paracetamol crystallized from solution (batch 1). *Arrows* indicate characteristic peaks of ethanol

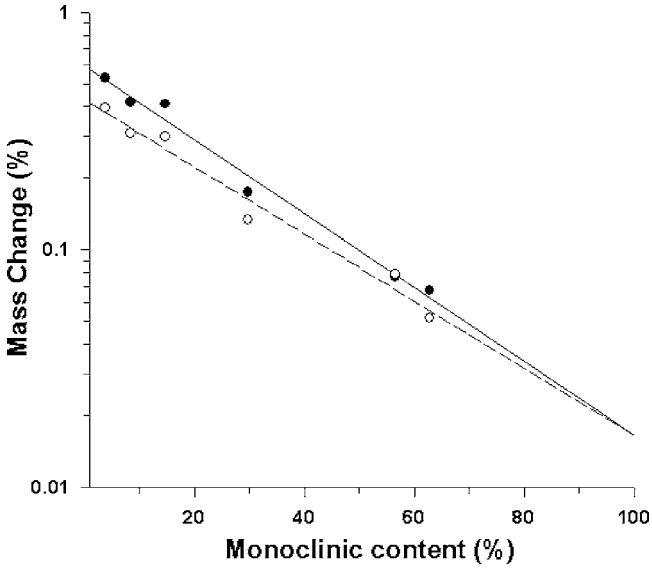

Fig. 6. Mass change *vs* monoclinic content of paracetamol form II crystallized from ethanol and corresponding linear regression lines, determined by gravimetric moisture sorption (*full symbols*, *solid line*), and by TGA (*empty symbols*, *dashed line*)

the crystals grown from solution greatly accelerates the transformation in comparison to the crystals grown from the melt and their physical mixtures with form I. It should be noted that the total mass change is slightly smaller than that determined at the end of a full RH cycle (Fig. 4a–b), probably because of some adsorbed moisture at the high relative humidity conditions. The comparison of the curves of batch 1 at 85% and 90% RH shows that the transformation advances with the same high rate at the beginning but becomes slower at the lower relative humidity towards the end of the process. So, grinding strongly accelerates the kinetics of the moisture induced transition reaction, but clearly does not remove the residual solvent.

Time-resolved PXRD patterns of intact and ground solution-grown form II crystals, recorded at 90% RH (Fig. 8a,b), reveal that the moisture induced transformation

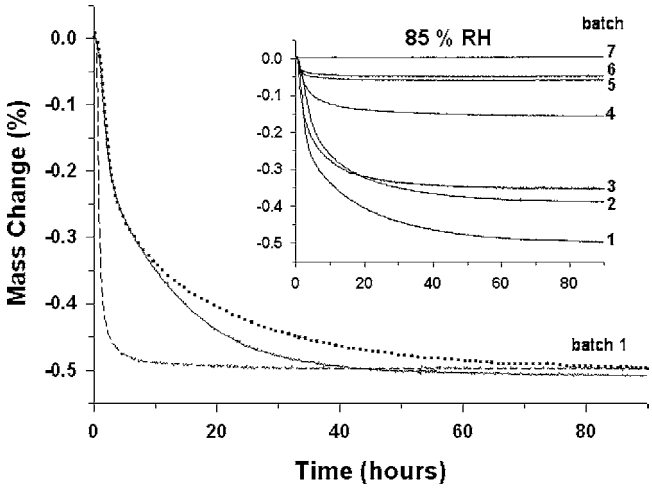

Fig. 7. Mass change of the different paracetamol form II samples obtained from ethanol solutions (batches 1–7) at 85% RH (*insert*), and of batch 1 at 85% (*dotted line*) and at 90% RH, intact (*solid line*) and ground (*dashed line*)

kinetics to form I is in very good agreement with the gravimetric studies. The reaction can be monitored by the decrease of the intensity of the 10.28° 2θ (002) and 12.70° 2θ (102) form II reflections and the simultaneous increase of intensity of the 12.09° 2θ (110) and 13.88° 2θ (001) reflections of form I. For the intact crystals (Fig. 8a), strong variations in peak intensities are observed, that can be explained by the rearrangement of the rather large crystals during the transformation process (extensive cracking along the slip plane). These variations could be minimized by grinding of the crystals (Fig. 8b), which is connected with a strong acceleration of the phase transformation kinetics. It is remarkable that some unexplained reflections appear transiently at low angles. The most prominent of these reflections are found at 13.58 and 13.77° 2θ, very close to the reflection of the (001) Miller plane of form I, at 13.88° 2θ. These high intensity peaks appear immediately after exposure of the crystals to high RH and persist almost throughout the transformation. Two rather weak reflections appear at 11.58 and 11.77° 2θ, close to the reflection of the (111) Miller plane of form I at 12.09° 2θ. Even after inducing complete transformation by heating at 140°C for 15 min, the 11.58 and 11.77° 2θ reflections do not quite vanish, while the 13.58° 2θ reflection shifts to 13.35° 2θ and increases in intensity. Another unexplained reflection of relatively high intensity appears at 15.31° 2θ, but very soon is masked due to the broadening of the growing 15.48° 2θ (−201) reflection of form I. Finally, some low intensity reflections appear slightly lower than the 10.28° 2θ (002) reflection of form II. Thus, it is reasonable to assume that these reflections derive from an unstable, new intermediate phase of paracetamol since the reflections do not match with any of the known phases including the hydrates. Alternatively, it could be that these reflections originate from distortions of the crystal lattice of the transforming crystals, since it is a single crystal to single crystal transformation that preserves the overall morphology of the original form, but the – initially – form II crystal undergoes significant expansion due to the lower density of the emerging form I. This point needs further investigations, but it is beyond the scope of the present study.

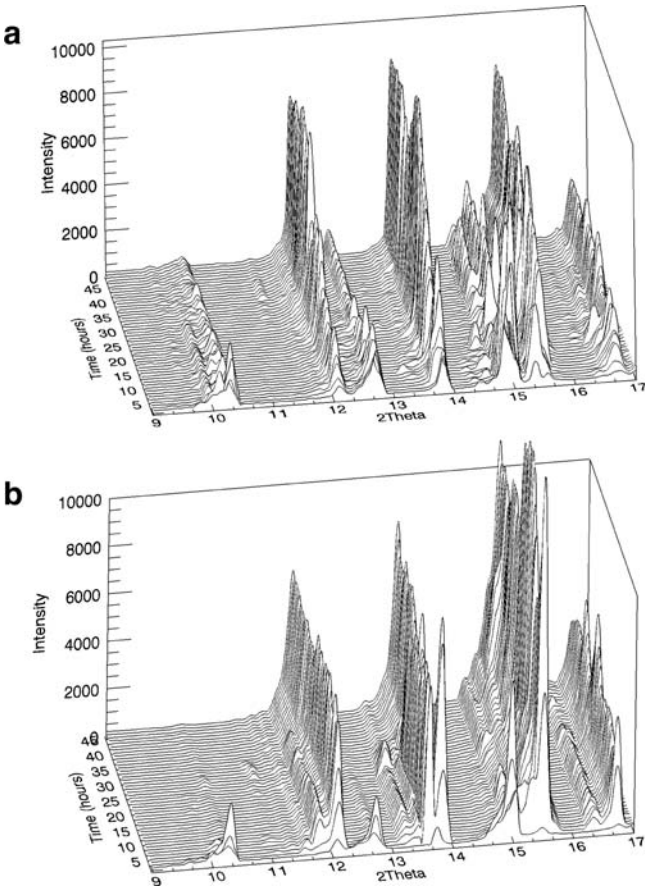

Fig. 8. Time-resolved PXRD patterns of intact (**a**) and ground (**b**) batch 1 at 90% RH, recorded sequentially every 60 min

Since the mass loss proceeds simultaneously with the transformation to form I, the transformation kinetics can be indirectly described by the mass loss *versus* time curves. Therefore, the percent mass loss determined at 85% RH was converted to transformed fraction, α, and the local values of the Avrami exponent, $n_{(\alpha)}$, which represent the mechanism of transformation (23), were calculated using Eq. 1 and plotted *versus* α (Fig. 9) according to Calka and Radlinski (22). The value of $n_{(\alpha)}$ does not remain constant, but rather changes in a manner depending on the initial ethanol content and the treatment (grinding) of the crystals. In general, the $n_{(\alpha)}$ values indicate that nucleation events take place over a limited time period at the beginning of the transformation, and then growth of the new form proceeds from a constant number of formed nuclei, consistent with a single-crystal-to-single-crystal transformation. More specifically, for the samples with a high ethanol and low initial form I content (batches 1–3), the Avrami exponent values are initially approximately 2.5, indicating a limiting case between constant and increasing nucleation rate. In the course of transformation, $n_{(\alpha)}$ continuously decreases until $\alpha \sim 0.5$ indicating a rate decrease and finally a cease of nucleation, and acquires a rather constant value of ≤ 1, that corresponds to growth of large particles (23). For the samples with high initial form I and low ethanol content (batches 4–7) $n_{(\alpha)}$ is initially ≥ 4 indicating a high nucleation rate which progressively decreases giving values of 1 at $\alpha \sim 0.6$. Grinding strongly affects the transformation mechanism, as $n_{(\alpha)}$ remains high for a longer time indicating that the transformation proceeds with a fairly high nucleation rate almost until the end. This is expected, since grinding induces defects on the crystals that can act as nucleation sites, and furthermore, it increases the surface area available for contact with moisture and for the departure of ethanol. This behavior argues clearly for a surface nucleation rather than bulk nucleation.

Observations conducted on a hot-stage microscope in a dry preparation (Fig. 10a–c) or in a high-melting-point silicone oil suspension (Fig. 10d–f), as well as in a controlled-atmosphere microscope stage (Fig. 10g–i), provided further insight into the transformation mechanism and the role of residual ethanol. In agreement with previous reports for orthorhombic crystals grown from ethanol solutions (14), the transformation follows a single-crystal-to-single-crystal mechanism, unlike the single-crystal-to-polycrystalline mechanism followed by crystals grown from water solutions (11). This further supports the view that ethanol is included in the lattice at the molecular level by a solid solution mechanism, because if liquid ethanol inclusions were present, they would

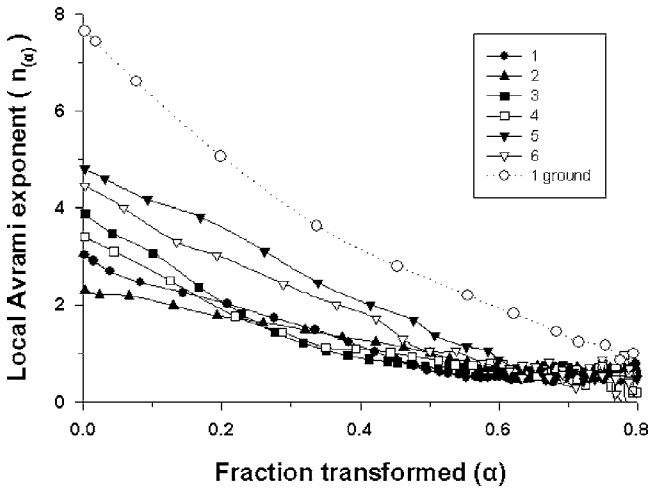

Fig. 9. Local Avrami exponents, $n_{(\alpha)}$ *versus* transformed fraction, α, for the moisture-induced transformation at 85% RH of the crystalline samples obtained from ethanol solutions (values for $\alpha>0.8$ are not shown because of noise)

provide multiple nucleation sites, leading to a polycrystalline mass after transformation, as is the case for the inclusions of water (11). The thermally induced transformation is initiated at defect sites (Fig. 10a–f) and occurs within the temperature range of 70–140°C with random distribution of the transformation temperature of individual crystals. A smooth interface front can be observed, propagating mostly diagonally, in agreement with previous reports for the transformation of orthorhombic crystals grown from ethanol solutions (28). Cracks form parallel to the (002) face (Fig. 10b,h) i.e. perpendicular to the *c* axis (along slip planes) and bubbles appear simultaneously, indicating the release of ethanol vapor (Fig. 10f). In the case of agglomerated crystals, the interface front propagates through the contact points, Fig. 10h, indicating a seeding effect, in agreement with the finding that the presence of form I accelerates the transformation. A similar pattern was observed for the moisture-induced transformation at 95% RH, Fig. 10g–i. Release of ethanol from the lattice seems to be necessary for – or at least to enhance – the creation of crystal defects or "microcavities" (29), that serve as nucleation sites. The overall behavior of the transforming crystals is compatible with the mechanism of "atypical" polymorphic transitions described by Mnyukh *et al.* (30). According to this mechanism, the uniform direction of the interface front can be explained as a result of epitaxy on the glide plane of the transforming polymorph.

The results of this study and previously reported data (11,14) stress the need to consider and study the potential effects of residual solvents on the kinetic stability of metastable polymorphs. As demonstrated, melt grown orthorhombic paracetamol is rather durable compared to samples crystallized from solvents, but always contains thermal decomposition products. A crystallization procedure of this metastable polymorph from solvents that results in a highly

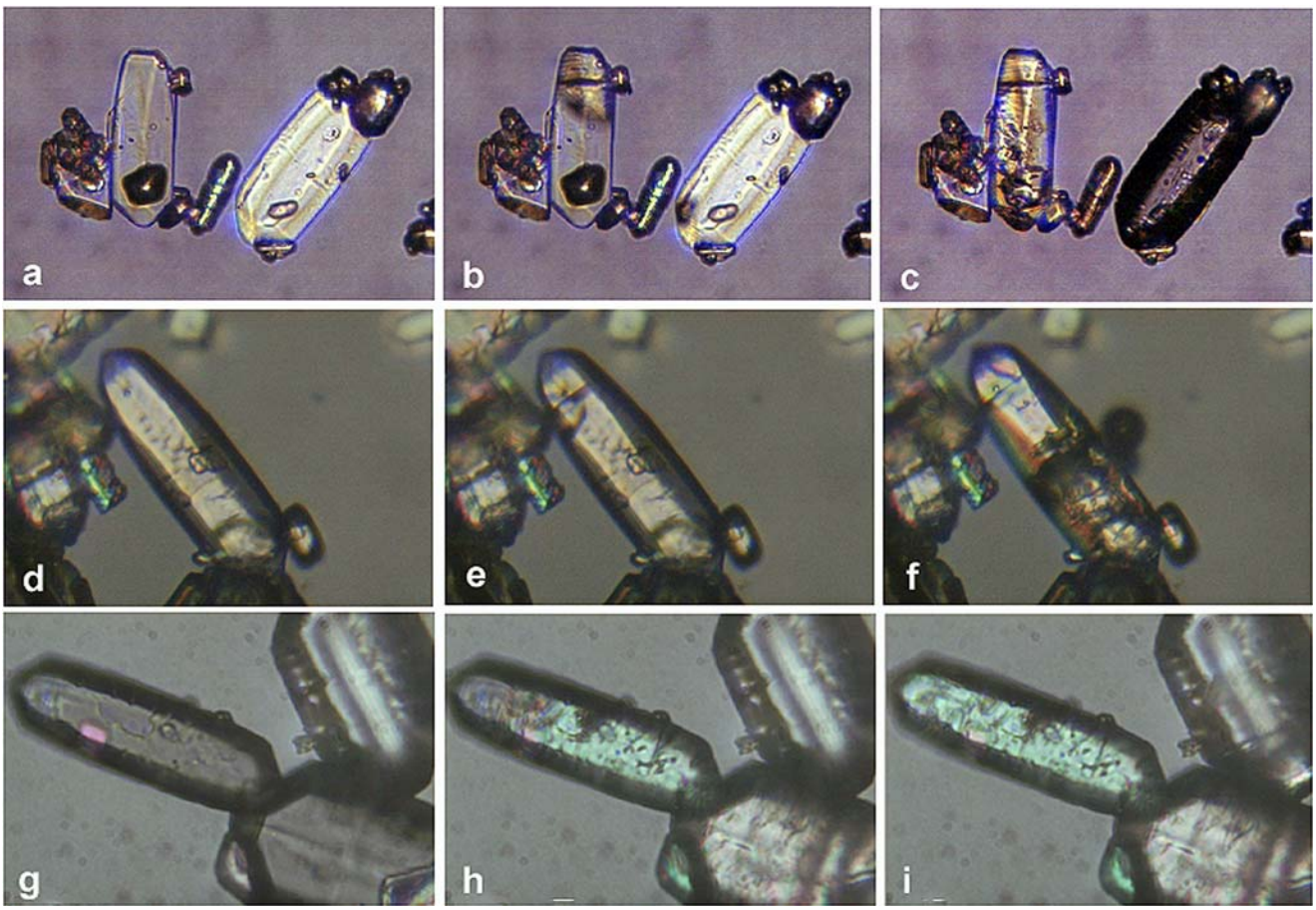

Fig. 10. a–i Photomicrographs of form II crystals undergoing transformation induced by heating (**a–f**), and by moisture (**g–i**)

pure and residual solvent free product seems to be the key for the development of stable tablets of this directly compressible polymorph. It should be emphasized that current pharmaceutical regulations on the topic residual solvent are strictly limited to solvent toxicity. According to the ICH Q3C guideline (31), water, which was proven to be responsible for the transformation of orthorhombic paracetamol grown from water solutions (11), is not considered a residual solvent, while ethanol, which was found in the present study to greatly accelerate the transformation of orthorhombic paracetamol, is a class 3 solvent with no specified acceptable limit. Anyway, this study highlights at a different aspect of the residual solvents problem and reveals the complexity of this phenomenon. We have to admit that the mechanism of residual solvent inclusion in pharmaceuticals is not a very well explored area (32). In order to get this issue under control we need to know whether the solvent is part of the crystal structure (33,34), present in amorphous or low ordered regions or mainly physically entrapped in voids/defects (liquid inclusions) of the crystals (35). It is obvious that the knowledge of the crystal structure is very helpful for such an evaluation.

CONCLUSIONS

The transformation of form II to the stable form at high relative humidity is accelerated by the presence of form I nuclei, as well as by grinding of the crystals. Solution-grown form II undergoes a mass loss of 0.1–0.6% *w*/*w* upon transformation due to the release of ethanol incorporated as residual solvent, most probably by a solid solution mechanism and not a physical liquid inclusion. The amount of residual ethanol is approximately inversely proportional to the initial monoclinic content of the samples, and it accelerates the moisture-induced transformation. Thus, it can be concluded that the moisture triggers a residual ethanol-mediated growth of existing form I nuclei but exerts a weaker effect on the nucleation of form I. The example of orthorhombic paracetamol highlights the significance of residual solvent incorporation in pharmaceutical crystals and its impact on the kinetic stability of metastable polymorphs. Furthermore the study demonstrates the outstanding analytical potential of gravimetric moisture sorption/desorption studies in the characterization of pharmaceuticals.

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