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Thermal analysis of paracetamol polymorphs by FT-IR spectroscopies

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ABSTRACT

A simple IR spectroscopy based methodology in routine screening studies of polymorphism is proposed. Reflectance and transmittance temperature-dependent IR measurements (coupled with the 2D-IR data presentation and the baseline analysis) offer a positive identification of each polymorphic phase, therefore allowing simple and rapid monitoring of the measured system. Applicability and flexibility of the methodology was demonstrated on the measurement of the model polymorphic compound paracetamol under various conditions (including geometric constraints and elevated pressure). The thermal behavior of paracetamol strongly depends on slight variations in experimental conditions that can result in formation of various phases (three polymorphs and the amorphous form). The amorphous phase can crystallize during heating into either Form II or Form III within almost identical temperature range. Likewise, the crystal transformations II → I and III → II also can proceed within almost identical temperature range. Furthermore, the thermal behavior is even more diverse than that, and includes the crystallizations of Forms I, II and III from the melt, and the high temperature II → I transition. The variety of the temperatures of the transformations is a major obstacle for unambiguous identification of a particular phase by DSC and a major reason for the implementation of these IR methods.

1. Introduction

Different crystal modifications of the same molecule (polymorphs) exhibit variations in solubility, stability, optical properties and melting temperatures that can cause far reaching ramifications on their production and applications. These considerations are of particular importance to pharmaceutical industry since the solid state of an active pharmaceutical ingredient (API) can drastically alter drug bioavailability [1]. Crystallization of a particular solid state can depend not only on temperature and pressure, but also on preparative conditions such as surface properties and trace quantities of impurities. For all these reasons there is a constant need for simple and reliable analytical methods in routine screening studies of the API polymorphism. In order to demonstrate applicability and flexibility of reflectance and transmittance temperature-dependent infrared measurements, these methods were employed in the present research of the model API polymorphic compound paracetamol.

Although analgesic and antipyretic compound paracetamol (acetaminophen, *N*-acetyl-4-aminophenol) is often cited as a model polymorphic system, some ambiguities regarding its solid-state transitions still remain. The three known polymorphs of paracetamol are well described regarding the crystal structures [2–5] and the vibrational spectra [6–12]: thermodynamically stable Form I (monoclinic, $P2_1/a$), metastable Form II (orthorhombic, *Pcab*), and obscure Form III (orthorhombic, $Pca2_1$) that is stable only under strict geometric constraints [5,6,8,9,13]. Even though solid-state transitions of these forms were investigated by extensive pressure-dependent [14] and atmospheric-dependent measurements [15], the thermal analytical (TA) methods where employed overwhelmingly, as is usually the case in the investigation of polymorphism [6–9,13,16–20]. The standard approach is based on the application of differential scanning calorimetry (DSC) aiming at proving the very existence of physical transition by measuring its temperature and the amount of heat exchanged. However, since such physical state measurements seldom reveal structural state changes coincident with temperature-dependent behavior, other more time-consuming methods are regularly employed (X-ray diffraction, vibrational and nuclear magnetic resonance spectroscopy, thermal microscopy, etc.). Conclusions based on separate physical and structural state measurements reveal inherent uncertainties due to the decoupled experiments, and therefore a method that can simultaneously provide the temperature of phase transition and information on the phases involved would be of outmost importance.

Vibrational techniques have proven their worth not only in identification of API polymorphs but also in qualitative and quantitative analyses of their powder mixtures [10,12,21]. Given that

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sample dissolution is not required these methods are economical, simple and rapid. Our earlier research has already demonstrated that FT-IR spectroscopy could also be used on its own as a stand alone alternative to DSC and thermal microscopy measurements by obtaining simultaneously phase transition temperature and structural information accompanying the transition [22]. Although in theory the appearance of the IR spectrum depends only on molecular absorptions, the recorded IR spectrum is always a combination of molecular and macroscopic properties of a measured sample. Baseline analysis for instance is primarily based on monitoring optical properties of the KBr sample pellet by temperature-dependent transmittance FT-IR in order to obtain transition temperatures (although one can also depict structural information with the careful analysis of these IR spectra). Even the slight variation of a compound physical properties leads to alteration in transmission, reflection and scattering of IR light, and consequently to the detection of solid-state transition temperature. Alongside transmittance IR measurements, we have also implemented temperature-dependent reflectance IR measurements in this work (single-reflection attenuated total reflectance (ATR) mid-IR spectroscopy). ATR technique is especially suitable for measurement of polymorphism since it enables spectrum acquisition without any sample preparation. Both IR methods enable acquirement of extensive range of spectral data, and the employment of two-dimensional correlation methods (2D-IR) greatly enhances comprehensibility of these large data sets [23].

It is remarkable that paracetamol, one of the oldest and the most widely available API whose solid-state conversions have been studied extensively to these days, can still cause strong disagreements within scientific community. The principal dispute remains over the solid-state transformation of Form II into Form I (II → I), and regarding the great commercial potential of Form II this is far from purely academic importance [6,9,15–17]. The present study of paracetamol polymorphs clearly demonstrated that here described TA IR techniques and chemometric methods (2D-IR) enable equivalent or superior results when compared with other recently published measurements [8,9,13,17,20]. Though this approach shares great similarities with the recently published method of coupled Raman-DSC system [9], it is more tolerant regarding the sample properties (for example, no fluorescence issues) and more often available than Raman spectroscopy. This study demonstrated not only the wide-ranging potential of the FT-IR thermal analysis for the future studies of polymorphism, but it also clarifies some uncertainties that still surround the phase transitions of paracetamol.

The new IR based methodology (baseline and MW 2D-IR analyses) for studying polymorphism is described in the first part of the report, while the second part describes in detail the thermal behavior of each paracetamol phase. As opposed to previous studies that were focused on only a partial polymorphic behavior of paracetamol (i.e. either on II → I or on III → II phase transition), this study has more comprehensive scope. To begin, it must be stated that the results of the study are in excellent agreement with previous measurements [5–9,13,15–18,20,24].

2. Materials and methods

2.1. Materials

Paracetamol was purchased from Aldrich. In order to eliminate influence of the thermal history of purchased sample, all paracetamol samples used in the measurements were obtained by heating the purchased sample to 190 °C, cooling the melt down to room temperature (r.t.) and reheating the amorphous paracetamol. The obtained solid forms (crystal and amorphous) were identified by their IR spectra according to the previously published research [6,8,10,12]. Form I was obtained by recrystallization of the melted sample during the cooling process either in a KBr pellet or between the ATR crystal and the sapphire anvil. Form II was obtained by reheating the amorphous form from r.t. up to 80 °C either on a pure KBr pellet or on the uncovered ATR crystal. Form III was obtained by reheating the amorphous paracetamol from r.t. up to 65 °C either between two pure KBr pellets or between the ATR crystal and the glass slide.

2.2. Methods

2.2.1. FT-IR measurement

FT-IR spectra were recorded at resolutions of 4 cm^{-1} on an ABB Bomem MB102 single-beam spectrometer, equipped with CsI optics and DTGS detector. Transmittance measurements were carried on a Specac 3000 Series high stability temperature controller with heating jacket. Reflectance measurements were carried on a Specac High Temperature Golden Gate ATR Mk II. Measurements were performed within the temperature range 25–190 °C under atmospheric conditions and at different heating/cooling rates (2–10 K min^{-1}). Each single-beam spectrum collected in one temperature run was ratioed to the single-beam spectrum of the sample-free setup (the reference spectrum) recorded immediately before starting the temperature-dependent measurements. The spectra were recorded with either a total of 30 scans (temperature resolution of one spectrum per 4 °C for a standard heating rate of 2 K min^{-1}), or a total of 6 scans (temperature resolution of one spectrum per one degree Celsius for a standard heating rate of 2 K min^{-1}).

The KBr sample pellets were prepared either by mixing ~2 mg of the paracetamol sample with 100 mg of KBr with a pestle and mortar or by applying the sample on the surface of pure KBr pellet. After completing the heating and cooling cycle some of the measured pellets (those with the paracetamol sample already embedded within the KBr matrix) were subsequently powdered with a pestle and mortar and recast into pellets for repeated measurements.

2.2.2. Data treatment

The baseline analysis designates an analysis of the baseline variations obtained from the raw (as-recorded data) temperature-dependent transmittance IR spectra at an arbitrarily chosen wavenumber assumed to be free of sample absorption (most often at around 2000 cm^{-1}). A simple plot showing transmittance (absorbance) at a given wavenumber versus temperature is found to be sufficient for obtaining phase transition temperatures.

The 2D correlation analysis was performed by means of discrete Hilbert transformation (as defined by Ozaki and Noda [23]) implemented in a program written for the setting of Matlab 6.5 (The MathWorks, Inc., Natick, MA). Moving window (MW 2D-IR) correlation maps were obtained as defined by Šašić et al. [25] and the 2D contour plots were generated as described by Thomas and Richardson [26]. Detailed description of the MW method and the baseline analysis can also be found in the previously published paper [22].

Qualitative and quantitative analysis of the ATR spectral data involved spectral searching performed by a home-made program based on root mean square error analysis written for the setting of Matlab 6.5. The spectral library contained two derivative ATR spectra collected at different temperatures for each of the five paracetamol phases (three crystal forms, the amorphous phase and the melt). The sample spectrum (the analyzed spectral region 1750–770 cm^{-1}) at a given temperature was compared with linear combinations of two library spectra (for every linear combination the proportion range of the first component was 1–100% (with 1% step)).

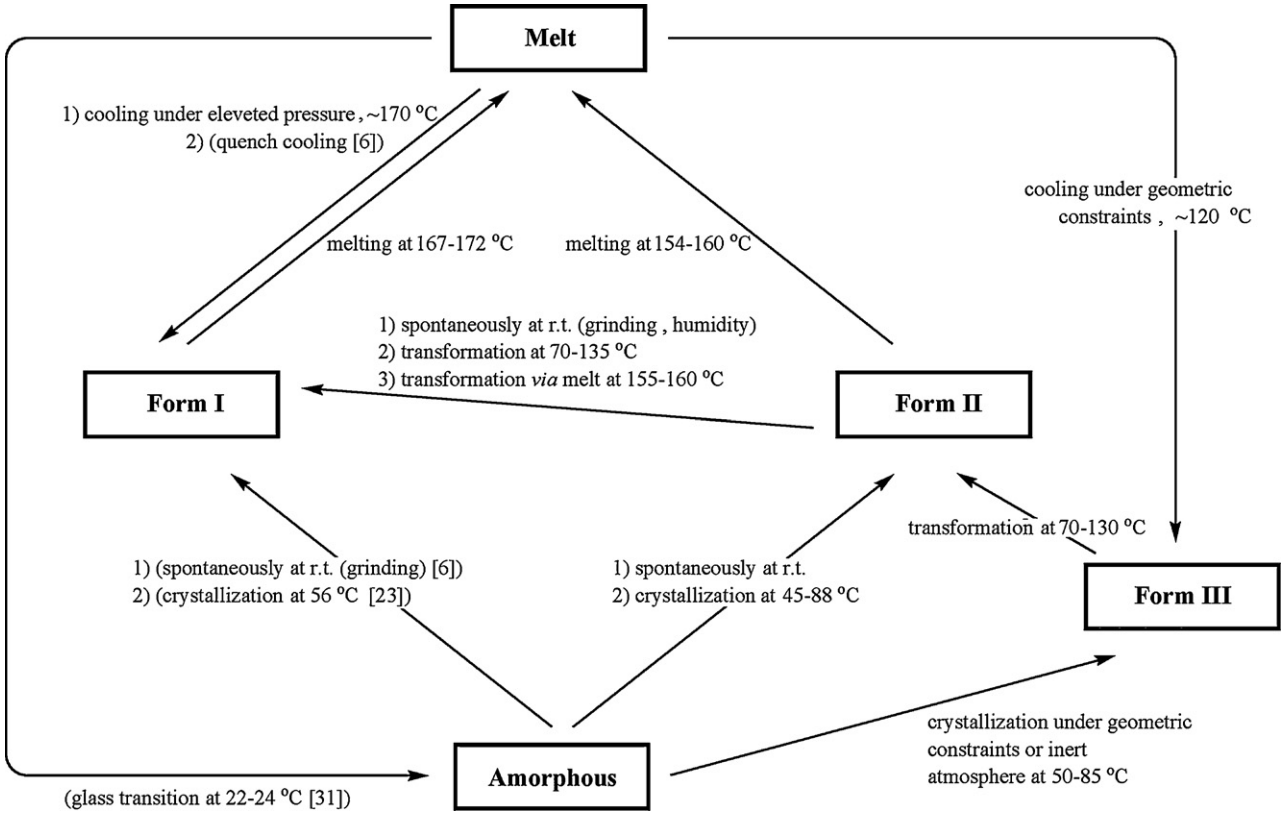

Fig. 1. Paracetamol phase transitions; up (down) arrows denote heating (cooling) processes. The temperature values are based on our measurements and the following Refs. [5–9,16,17,19,21,22,24,27]. Additional transformations that were not observed in this research are cited in brackets.

3. Results and discussion

The measurements were conducted using only temperature-dependent IR spectroscopy without simultaneous or parallel DSC measurements. Therefore, the reported paracetamol polymorphs and their transition temperatures are based exclusively on spectral appearances and temperature-dependent spectral changes. A transition temperature value is defined as the onset of spectral changes. The complete list of the paracetamol phase transitions and the corresponding IR spectra is presented in Fig. 1 and the Supplementary data (Figs. S1–S10).

3.1. Baseline analysis of the paracetamol phase transitions

As opposed to other TA infrared spectroscopies that are based solely on vibrational band changes the baseline analysis monitors temperature-dependent baseline variations. Thereupon, it uses only those parts of a FT-IR spectrum that are free of molecular absorptions (most often at around 2000 cm^{-1}). Since high spectral resolution is not imperative, it is possible to make spectral acquisition times shorter by lowering the spectral resolution and by applying faster heating rates equivalent to those in DSC experiments, without the loss of temperature resolution (the temperature resolution in this study was 1 °C per one spectrum). If some structural transformation is determined within the available temperature range, more thorough IR search for structural information can be made by applying better spectral resolution. The baseline analysis capability was demonstrated on differently prepared paracetamol samples (Fig. 2).

The simplest case represents melting of Form I within the KBr pellet (Fig. 2a). Heating of gently grinded Form II within the KBr pellet shows more curious behavior (Fig. 2b). Physical separation of the Form II crystal domains within the KBr matrix creates experimental conditions that allow II → I transformation of some Form II domains (~110 °C) while others remain stable and melt above 155 °C, followed by melting of Form I above 170 °C. Although, such thermal behavior was not previously observed by means of vibrational techniques, analogous behavior was observed using thermomicroscopy [16]. Based on the published method for identification and quantitation of Form II and Form I in powder mixtures

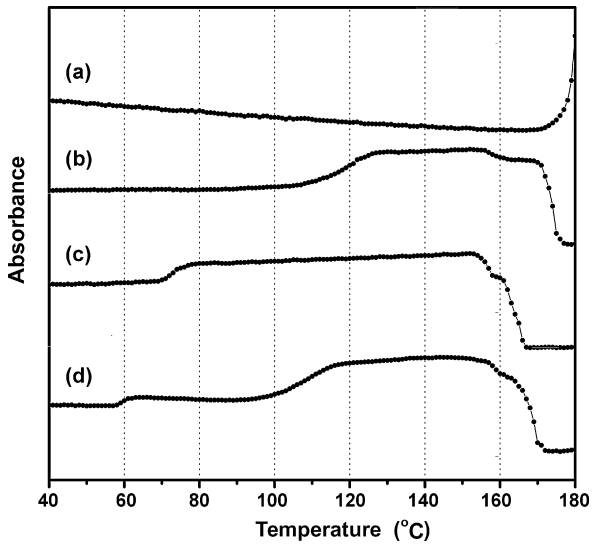

Fig. 2. Baseline variations at 2000 cm^{-1} of the paracetamol samples recorded under different conditions: (a) melting of Form I within the KBr pellet, (b) heating of Form II within the KBr pellet, (c) reheating of the amorphous paracetamol on the KBr pellet, (d) reheating of the amorphous phase between KBr pellets; heating rates 2 K min^{-1}. For better viewing the absorbance values are scaled and offset.

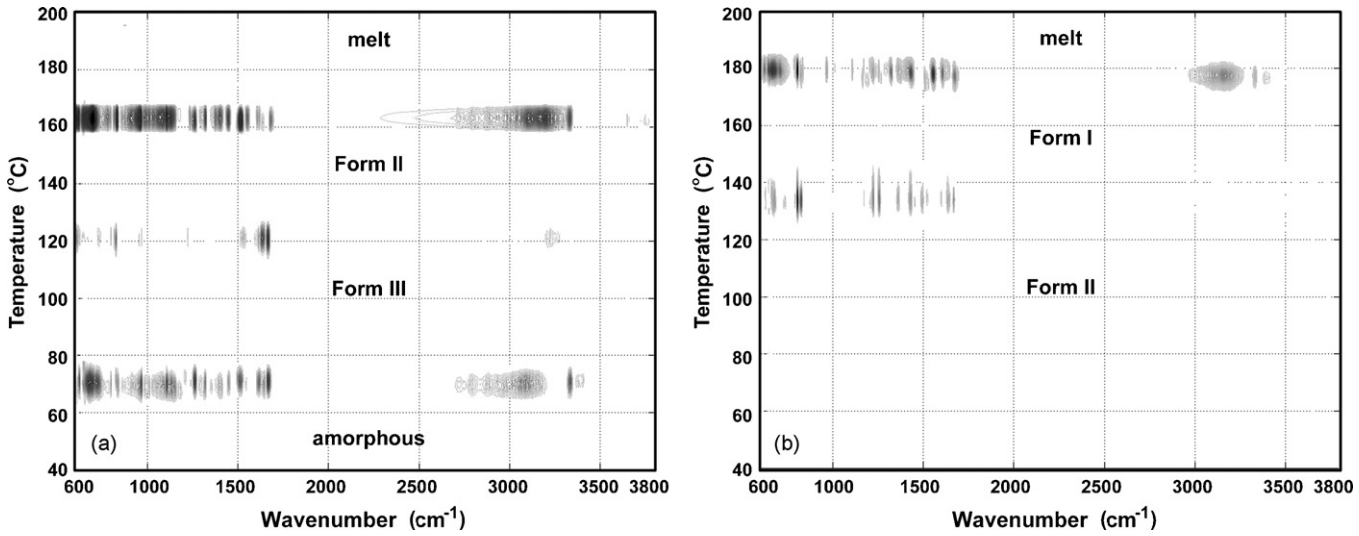

Fig. 3. MW 2D-IR correlation analysis of the ATR spectral data: (a) reheating of the amorphous phase between the ATR crystal and the glass slide, (b) heating of Form II between the ATR crystal and the sapphire anvil. Analyzed spectral region: 600–3800 cm^{-1}; window size: 10 spectra; window step: 1 spectrum; heating rate: 2 K min^{-1}; resolution: 4 cm^{-1}; number of scans per spectrum: 6.

[10], the approximate quantity of transformed Form II in the pellet (depicted in Fig. 2b) was 65%. This cannot be reproduced in single-reflection ATR measurements because spectra are acquired from a single crystal domain of approximately 0.5 mm in diameter, while the transmittance method obtains spectra from a multitude of crystal domains separated within KBr pellet.

The crystallization of Form II (70 °C) and the subsequent melting (155 °C) was obtained by reheating the amorphous paracetamol on the pure KBr pellet (Fig. 2c). By reheating the amorphous phase between two pure KBr pellets resulted in distinctive thermal sequence: amorphous → III → II → melt (phase transition temperatures: 58 °C, 100 °C, 157 °C) (Fig. 2d). It is interesting that the baseline analysis detected certain interruption in the melting of Form II at approximately 160 °C (distinct shoulder in Fig. 2c and d). Above the melting temperature of Form II the system can still recrystallize as Form I providing that the heating rates leading towards the melting temperature of Form I are slow enough (this behavior is discussed in detail in Section 3.6). In the presented baseline analysis the heating rate of 2 K min^{-1} is too fast for emergence of any IR detectable quantity of Form I (less than 0.012 mole fraction, according to the published data [10]). However, it is reasonable to assign this interruption in the melting to the equilibrium process between Form I nucleation and isotropic liquid (melt).

3.2. MW 2D-IR analysis of the paracetamol phase transitions

FT-IR spectrometers provide rapid acquisition of a large amount of spectral data. In the heating processes of this study (25–190 °C) the temperature-dependent IR spectra were collected with the temperature resolution of 1 °C per one spectrum. The determination of phase transition temperatures and the chemical structures accompanying it from such huge data sets is facilitated by the implementation of moving window (MW) 2D-IR data representation. An MW contour map shows temperature-ordered spectral changes and thus present temperatures of phase transitions and associated structural information. It is important to emphasize that signals in the contour map are directly related to spectral changes. In other words, the temperature interval devoid of spectral changes is present as a blank area therefore indicating the temperature range of the presence of only one phase. In contrast, peaks of the contour map are found at temperatures where the largest spectral variations occur therefore revealing the presence of a phase transition.

The contour maps of the two most debated thermal histories of paracetamol are depicted in Fig. 3. A heating process of the cooled amorphous paracetamol under strict geometrical constraints (between ATR crystal and cover glass slide) is shown in Fig. 3a. The phase transitions starting with crystallization of Form III (65 °C), III → II transformation (117 °C), and finally melting of Form II are evidently present in the contour map. In an equally unambiguous fashion the heating process of Form II (between the ATR crystal and the sapphire anvil) with the low temperature II → I crystal-to-crystal transformation (128 °C), followed by melting of Form I above 172 °C is presented (Fig. 3b). In the third type of experiment, Form II was obtained by reheating the amorphous form on the uncovered ATR crystal from r.t. up to 80 °C, followed by melting of Form II at 158 °C.

3.3. Qualitative and quantitative IR analysis of the paracetamol phase transitions

Alternatively, these spectral data sets can be processed by applying qualitative and quantitative analysis based on the root mean square error minimization (described in Section 2). As opposed to multivariate analysis of the spectral data [9], this analysis is based on the reasonable assumption that the measured system is either in homogeneous single-phase state or in two-phase equilibrium. It must be stated that the sole purpose of this rough model is monitoring of temperature-dependent phase transitions and detection of transition temperatures and not a quantitative analysis of polymorph mixtures (qualitative and quantitative vibrational measurement of polymorph mixtures of paracetamol was successfully achieved in the previous studies [10,12]). The analysis of the two data sets (Figs. 3 and 4) indicates that a simple qualitative and quantitative estimate of the thermal process can be achieved in this way. The temperatures of phase transitions obtained by this method are as follow: (a) 63 °C (crystallization of Form III), 113 °C (III → II transformation) and 158 °C (melting of Form II), and (b) 123 °C (II → I transformation) and 174 °C (melting of Form I).

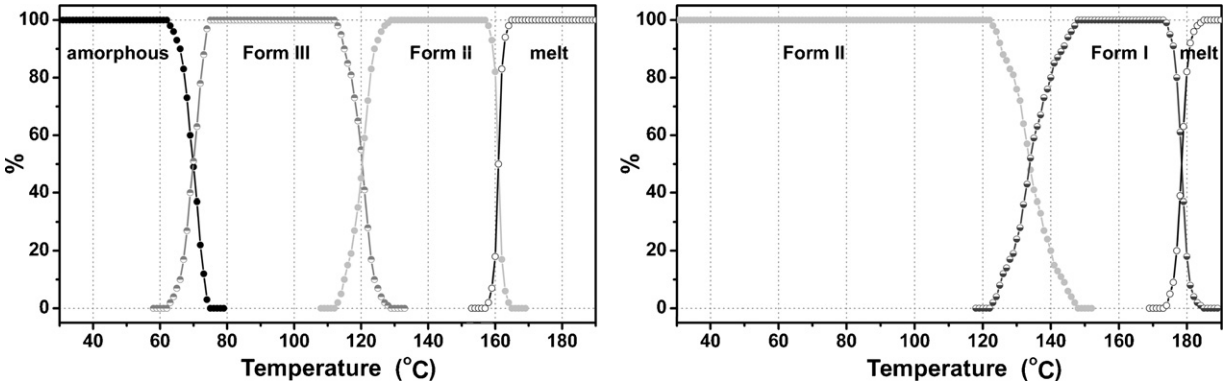

Fig. 4. Estimated percentile contributions of the paracetamol phases to the temperature-dependent spectral data.

3.4. Amorphous paracetamol

The crystallization of different polymorphs from the amorphous phase is not the only reason to investigate melted and amorphous phases. The amorphous form shows the most pronounced differences of physical properties to the thermodynamically stable crystal modification. Since it is the most soluble solid form, there is also a commercial reason for studying its stability and phase transformation [27]. Amorphous paracetamol is easily obtained by cooling a sample of Form I previously heated above the melting point (170 °C).

In all of the measurements a sample was heated to 190 °C and then cooled down to r.t. at various rates (2–10 K min^{-1}). As a rule the amorphous solid phase was obtained (several exceptions regarding crystallization of Forms I, II and III directly from the melt are reported below). When comparing IR spectra of the melt and the amorphous phase it is rather obvious that the spectral appearance and bands positions remained almost unchanged. However, the relative peak intensities changed significantly, with the most noticeable change regarding the peak at 1541 cm^{-1} (Fig. S10). Not surprisingly these spectral changes gradually occurred from 190 °C down to r.t. since the glass transition temperature is at the lower end of the measuring temperature interval (T_g = 23–26 °C).

3.5. Form I paracetamol

As previously stated in the materials section the monoclinic Form I can be obtained by cooling the melt under elevated pressure (either in KBr pellet or under the sapphire anvil). The recrystallization into the Form I starts at approximately 170 °C and proceeds quite rapidly (Fig. 5). In the subsequent heating run encompassing 25–190 °C temperature interval Form I does not show any phase transition other than melting. In the TA measurements that do not simultaneously obtain structural information (DSC) such behavior is often cited as indicative for thermodynamically stable Form I [24].

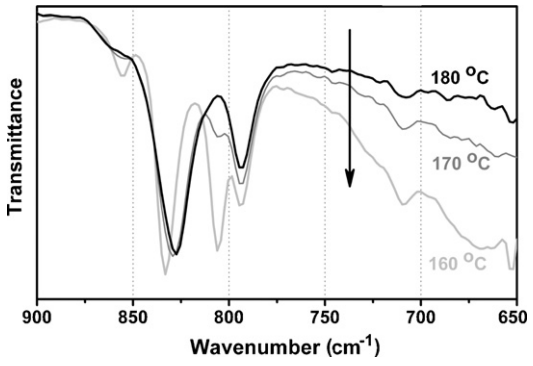

Fig. 5. IR spectra of the recrystallization of Form I (cooling of the melted paracetamol between the ATR crystal and the sapphire anvil).

3.6. Form II paracetamol

Phase transitions of orthorhombic Form II gained much attention in the last decade. The principal reason is the substantial commercial potential of Form II due to its suitability for tabletting by direct compression (as opposed to commercially used Form I) [28]. Studies have shown that Form II can be straightforwardly produced by heating of the amorphous phase and later used as a seeding material for crystallization from solvent [16]. However, the thermal behavior of Form II still puzzles scientific community, with the principal point of dispute being the exact nature of II → I phase transformation that was allegedly detected within the broad temperature interval 70–156 °C [6,9,15–17]. Although the existence of the direct II → I transition was indisputably proven by temperature-dependent X-ray measurements of single crystals [17], there are also numerous measurements of Form II melting at 155–160 °C (without prior conversion into Form I) [9,15,16], and even transformation into Form I above the melting temperature [6].

In this study Form II was regularly obtained by reheating of the solidified melt (amorphous phase). The exact temperature of crystallization varied from sample to sample and occurred within the range 55–80 °C, and these values are in accordance with those previously reported (i.e. within the range of 45–88 °C) [6,7,9,16]. The only exception was the crystallization of the amorphous paracetamol into Form II at the end of the cooling process (r.t.) in one measurement. Although spontaneous crystallization at r.t. is somewhat unusual it is in agreement with the previously published observations that the amorphous sample can crystallize into orthorhombic Form II [6,9].

Reheating the cooled Form II can result in three different ways of thermal behavior. If a measurement is conducted with relatively fast heating rates (≥2 K min^{-1}) the only observed phase transition is the melting of Form II at 155–160 °C (Fig. 6a). However, if the slower heating rates are employed (≤0.5 K min^{-1}) than the only observed phase transition is high temperature II → I transformation at approximately 155 °C (Fig. 6b). The formation of Form I can be easily determined by the appearance of a diagnostic peak at 806 cm^{-1} [10]. In the third case there is a two-phase phenomenon: (a) II → I transformation at lower temperatures, and (b) melting of Form I above 170 °C (Fig. 6c). The onset of this low temperature II → I transformation takes place within the range 95–125 °C (it slightly varies from sample to sample in accordance with previous observations [17]), and it generally occurs when the starting material was manipulated by either fragmentation or grinding. Such low temperature II → I transformation is most likely accel-

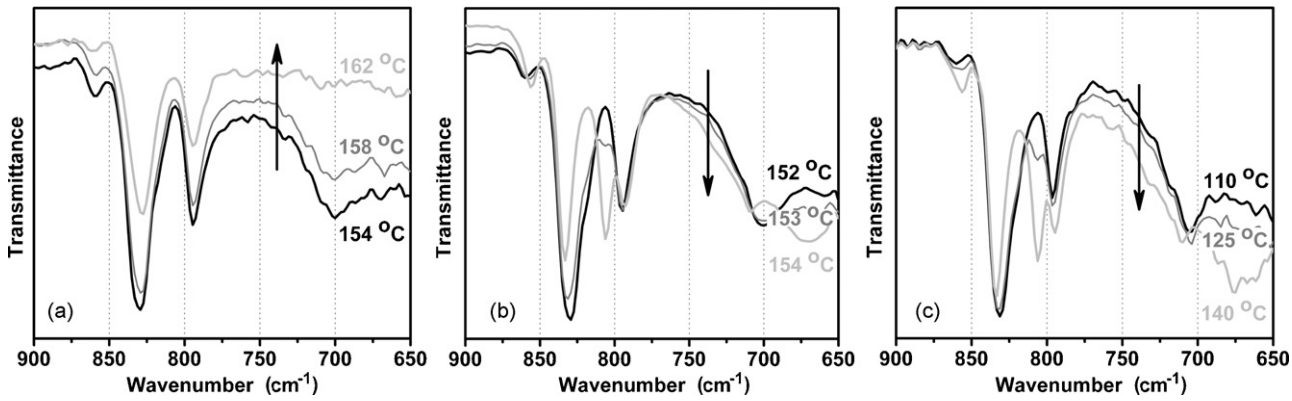

Fig. 6. IR spectra of the phase transitions of Form II during the various heating processes: (a) melting (heating rate 2 K min^{-1}), (b) high temperature II → I transition (0.5 K min^{-1}), and (c) low temperature II → I transition (2 K min^{-1}).

erated by humidity and the traces of solvent (the crystals of Form II grown from solution are especially susceptible to low temperature II → I transformation, as opposed to those obtained from the melt) [15–17].

Contrary to the direct II → I transition at lower temperatures, the high temperature transformation at 155 °C is probably not a straightforward crystal-to-crystal transition but it is rather preceded by melting of Form II and recrystallization of Form I. Such explanation was already put forward, but based on the temperature-dependent XRPD measurements [6]. It remained a point of strong disagreement [17]. No presence of the melted phase during II → I transformation was detected in our IR experiments (neither in the published XRPD measurements). It is, however, symptomatic that the spectral baselines (in the reflectance IR) behave differently at low and high temperature II → I transformations (Fig. 6b and c). The baseline variations at the high temperature II → I transformations are similar to those encountered for other crystal-to-melt and amorphous-to-crystal transitions of paracetamol. The spectral baseline in ATR measurements is predominantly the result of optical contact between the sample and the ATR crystal (what is seen in ATR is what is happening on the surface of the sample), and its different behavior can indicate that, although these two II → I phase transitions start and result with the same polymorphs, they proceed through different mechanism.

3.7. Form III paracetamol

Form III is the least stable known polymorph of paracetamol that can be isolated only under strict geometric constraints. Normally it is produced by melting of paracetamol, placing the sample between a slide and a cover glass, cooling it to r.t., and reheating up to 85 °C (the crystallization occurs within 50–85 °C interval) [5–9,13,20]. In our measurements this crystallization occurred regularly from 60 °C to 70 °C. At higher temperatures Form III undergoes crystal-to-crystal transformation into Form II within the temperature interval 100–120 °C, again well within the published interval (70–130 °C). Further heating resulted in melting of Form II at 155–160 °C, and no II → I transformation preceding the melting has ever been observed. This temperature behavior of paracetamol is in excellent agreement with the majority of previous measurements [5–9,13,20].

Among the published measurements there are only two cases with the results different from the generally accepted (also in this study) viewpoint. The first report described preparation of paracetamol polymorphs from binary mixtures containing 10% (w/w) of hydroxypropyl methylcellulose (HPMC) [19]. Although the authors concluded that Form III has transformed into Form I, the IR spectra and the DSC thermograms both suggest that such binary systems present more complicated thermal behavior than is the case of the pure paracetamol. Therefore, such a behavior most likely cannot be directly correlated to the paracetamol polymorphism. The only ever presented case of direct melting of Form III was described in another report [20]. The authors assumed that the Form III was obtained from amorphous paracetamol following the heating–cooling cycles in DSC pans, although there were no direct structural measurements to support that assumption. The measurements of (allegedly) Form III performed in hermetic pans under ambient atmosphere showed single melting transition at 156 °C, while the analogous measurements of amorphous samples showed only an additional exothermic transition in the 60–80 °C region. Based on the assumption that the measured system was indeed Form III the authors concluded that Form III melts at 155–156 °C (without prior transformation into Form II). It is important to emphasize that amorphous paracetamol can crystallize as either Form III or II within almost identical temperature range (50–80 °C), and for that reason the identity of crystallized phase remains ambiguous without some additional structural information that DSC cannot provide. Therefore, to claim solely on a DSC data that a particular polymorph has been obtained, particularly if that polymorph is metastable, might not always be justifiable. With that in mind, it is more reasonable to assume that Form II, and not Form III, was obtained in hermetic pans under ambient atmosphere, especially since the described thermal behavior depicts Form II perfectly. This premise is further corroborated with the subsequent measurements that obtained Form III only under N_2 atmosphere and never under air [5].

Although the Form III is reproducibly obtained by reheating the amorphous paracetamol, it seems that such approach is not the only viable option. In one of our measurements (out of the six conducted) the spontaneous recrystallization has occurred at approximately 120 °C during the cooling. Such an observation has never been reported before and was even considered improbable on account of previous measurements [13]. The cited premise by Burley et al. states that the main requirement for isolation of metastable Form III is hindrance of molecular movement that allows slow relaxation towards the crystalline state (and according to the Ostwald's rule of stages the least stable polymorph would be the first to crystallize). Though all previous measurements and a majority of ours demonstrated that the most reliable way for obtaining Form III is a gentle warming of the amorphous phase, here presented observation clearly shows that the spontaneous crystallization into Form III from the supercooled liquid is also possible. It seems that the important factor in successful isolation of Form III via the cooling route was relatively fast cooling rate (10 K min^{-1}) that prevented subsequent transformation of Form III into Form II (according to the published data III → II transformation is possible above 70 °C).

3.8. Potential of the FT-IR thermal analysis

Thermal analyses should provide three distinct information regarding particular phase transition: (1) identity of the phases involved (structural information), (2) phase transition temperature, and (3) heat value of the transition (or heat capacity of the system). While temperature-dependent structural techniques (XRPD, IR, Raman, NMR, thermal microscopy) can provide information about temperatures and structures, the DSC measurement can only provide temperatures and heat values. However, it must be stated that the important thermodynamic studies of paracetamol could only be made with meticulous DSC measurements [13,17,24,27,29,30]. Although it can appear that both approaches have equal drawbacks, it is quite obvious that the shortcomings of DSC are more severe on account that without the conclusive structural identification the other information are less valuable. In addition to that, some phase transitions, particularly crystal-to-crystal polymorph transformations have thermal outputs that can be below the detection limit of a DSC instrument. Both of these reasons can lead to erroneous conclusions when DSC is used on its own. This study clearly demonstrated that crystal-to-crystal transitions of paracetamol (with the associated enthalpy of only 0.3–1.2 kJ mol^{-1} in the broad temperature range [8,13,16]) are better detected by the temperature-dependent FT-IR spectroscopy than by traditional DSC measurements.

The complete set of information can be acquired with coupled vibrational-DSC measurements [8,9,18]. However, it is quite indicative that two of these paracetamol studies did not even reported DSC measured values of the heats of transition, but only the phase transition temperatures [9,18]. Considering that temperature-dependant vibrational measurements can provide accurate structural and temperature data on its own, it is quite unexpected that instead of exploring the full potential that this technique offers the measuring conditions were constrained in order to accommodate the DSC experimental conditions. Therefore, the two DSC/FT-IR microspectroscopy studies detected only a fraction of paracetamol phase transitions (namely, melting of Form I, and sequence amorphous → III → II → melt) [8,18]. In the case of Raman detected differential scanning calorimetry the whole point of coupled measurement is questionable since the heat values are strongly influenced by the laser radiation [9].

FT-IR thermal analysis has considerable advantages over thermal microscopy as well. While both of these TA methods enable structural monitoring of phase transitions, IR spectroscopy offers straightforward relationship between the sample at a certain temperature (i.e. its IR spectrum) and accompanying molecular structure (including intra- and intermolecular interactions) by means of spectra–structure correlations. This study revealed that flexibility of IR based TA allows measurement of a sample under variety of experimental settings, including arrangements that imitate thermal microscopy (sample between two pure KBr pellets or between the ATR crystal and the glass slide), as well as some that are out of reach of regular thermal microscopy (elevated pressure in a KBr pellet or between the ATR crystal and the sapphire anvil). As a result of this adaptability the two major disadvantages of the two FT-IR methods (grinding and compression into KBr pellet, and pressure exerted on the sample between the ATR crystal and the sapphire anvil) were successfully resolved (measurements on a sample deposited on a pure KBr pellet or on an uncovered ATR crystal). Variability of experimental settings is quite important in complete characterization of a sample because various processes (such as grinding and elevated pressure) can induce solid-state transitions on sensitive metastable polymorphs. Hence, owing to the highly adaptable experimental settings, this study perceived more varieties of paracetamol phase transitions than any previous study, including some transformations that were never before reported (crystallizations of Forms I and III during the cooling process). It should be stated, though, that such good results were facilitated by the stability of paracetamol melt and, therefore, measurement of a compound that decompose at temperature below melting should be more complicated.

4. Conclusions

The thermal behavior of paracetamol strongly depends on the slight variations in experimental conditions that can result in formation of various phases. The amorphous phase can crystallize during heating into either Form II or Form III (and supposedly Form I [20]) within almost identical temperature range (50–80 °C). The analogous remark applies to the crystal transformations II → I and III → II that also proceed within almost identical temperature range (70–130 °C). Furthermore, the range of transformation is even more diverse than that, including the crystallizations of Forms I, II and III during the cooling process, and high temperature II → I transition (above the melting temperature of Form II). These inconclusive temperatures of crystallization and transformation are a major obstacle for unambiguous identification of a particular phase by DSC.

FT-IR thermal analysis (coupled with the 2D-IR data presentation and the baseline analysis) offers a positive identification of each paracetamol phase, therefore allowing simple and rapid monitoring of the measured system. Besides clarifying some ambiguous thermal behavior of paracetamol, the study also presented the potential of FT-IR thermal analysis for the future studies of polymorphism.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2010.08.023.

References

[1] R. Hilfiker, F. Blatter, M. von Raumer, Relevance of solid-state properties for pharmaceutical products, in: R. Hilfiker (Ed.), Polymorphism in the Pharmaceutical Industry, Wiley-VCH, Weinheim, Germany, 2006, pp. 1–19.
[2] M. Haisa, S. Kashino, R. Kawai, H. Maeda, The monoclinic form of p-hydroxyacetanilide, Acta Crystallogr. B 32 (1976) 1283–1285.
[3] D.Y. Naumov, M.A. Vasilchenko, J.A.K. Howard, The monoclinic form of acetaminophen at 150 K, Acta Crystallogr. C 54 (1998) 653–655.
[4] T.N. Drebushchak, E.V. Boldyreva, Variable temperature (100–360 K) single-crystal X-ray diffraction study of the orthorhombic polymorph of paracetamol (p-hydroxyacetanilide), Z. Kristallogr. 219 (2004) 506–512.
[5] M.-A. Perrin, M.A. Neumann, H. Elmaleh, L. Zaske, Crystal structure determination of the elusive paracetamol form III, Chem. Commun. (2009) 3181–3183.
[6] P. Di Martino, P. Conflant, M. Drache, J.P. Huvenne, A.M. Guyot-Hermann, Preparation and physical characterization of forms II and III of paracetamol, J. Therm. Anal. Calorim. 48 (1997) 447–458.
[7] M. Szelagiewicz, C. Marcolli, S. Cianferani, A.P. Hard, A. Vit, A. Burkhard, M.V. Raumer, U.C. Hofmeier, A. Zilian, E. Francotte, R. Schenker, *In situ* characterization of polymorphic forms, J. Therm. Anal. Calorim. 57 (1999) 23–43.
[8] S.-L. Wang, S.-Y. Lin, Y.-S. Wei, Transformation of metastable forms of acetaminophen studied by thermal Fourier transform infrared (FT-IR) microspectroscopy, Chem. Pharm. Bull. 50 (2002) 153–156.
[9] J.F. Kauffman, L.M. Batykefer, D.D. Tuschel, Raman detected differential scanning calorimetry of polymorphic transformations in acetaminophen, J. Pharm. Biomed. Anal. 48 (2008) 1310–1315.
[10] N. Al-Zoubi, J.E. Koundourellis, S. Malamataris, FT-IR and Raman spectroscopic methods for identification and quantitation of orthorhombic and monoclinic paracetamol in powder mixes, J. Pharm. Biomed. Anal. 29 (2002) 459–467.
[11] E.B. Burgina, V.P. Baltakhinov, E.V. Boldyreva, T.P. Shakhtschneider, IR spectra of paracetamol and phenacetin. 1. Theoretical and experimental studies, J. Struct. Chem. 45 (2004) 64–73.

[12] B.B. Ivanova, Monoclinic and orthorhombic polymorphs of paracetamol – solid state linear dichroic infrared spectral analysis, J. Mol. Struct. 738 (2005) 233–238.
[13] J.C. Burleya, M.J. Duera, R.S. Steina, R.M. Vrcelj, Enforcing Ostwald's rule of stages: isolation of paracetamol forms III and II, Eur. J. Pharm. Sci. 31 (2007) 271–276.
[14] E.V. Boldyreva, T.P. Shakhtshneider, H. Ahsbahs, H. Sowa, H. Uchtmann, Effect of high pressure on the polymorphs of paracetamol, J. Therm. Anal. Calorim. 68 (2002) 437–452.
[15] K. Kachrimanis, K. Fucke, M. Noisternig, B. Siebenhaar, U.J. Griesser, Effects of moisture and residual solvent on the phase stability of orthorhombic paracetamol, Pharm. Res. 25 (2008) 1440–1449.
[16] G. Nichols, C.S. Frampton, Physicochemical characterization of the orthorhombic polymorph of paracetamol crystallized from solution, J. Pharm. Sci. 87 (1998) 684–692.
[17] E.V. Boldyreva, V.A. Drebushchak, I.E. Paukov, Y.A. Kovalevskaya, T.N. Drebushchak, DSC and adiabatic calorimetry study of the polymorphs of paracetamol, J. Therm. Anal. Calorim. 77 (2004) 607–623.
[18] S.Y. Lin, S.L. Wang, Y.D. Cheng, Thermally induced structural changes of acetaminophen in phase transition between the solid and liquid states monitored by combination analysis of FT-IR/DSC microscopic system, J. Phys. Chem. Solids 61 (2000) 1889–1893.
[19] A. Rossi, A. Savioli, M. Bini, D. Capsoni, V. Massarotti, R. Bettini, A. Gazzaniga, M.E. Sangalli, F. Giordano, Solid-state characterization of paracetamol metastable polymorphs formed in binary mixtures with hydroxypropylmethylcellulose, Thermochim. Acta 406 (2003) 55–67.
[20] S. Qi, P. Avalle, R. Saklatvala, D.Q.M. Craig, An investigation into the effects of thermal history on the crystallisation behaviour of amorphous paracetamol, Eur. J. Pharm. Biopharm. 69 (2008) 364–371.
[21] B.B. Koleva, T.M. Kolev, M. Spiteller, Determination of cephalosporins in solid binary mixtures by polarized IR- and Raman spectroscopy, J. Pharm. Biomed. Anal. 48 (2008) 201–204.
[22] B. Zimmermann, G. Baranović, Determination of phase transition temperatures by the analysis of baseline variations in transmittance infrared spectroscopy, Appl. Spectrosc. 63 (2009) 1152–1161.
[23] Y. Ozaki, I. Noda, Two-Dimensional Correlation Spectroscopy, John Wiley and Sons, Chichester, 2004.
[24] P. Espeau, R. Ceolin, J.L. Tamarit, M.A. Perrin, J.P. Gauchi, F. Leveiller, Polymorphism of paracetamol: relative stabilities of the monoclinic and orthorhombic phases inferred from topological pressure–temperature and temperature–volume phase diagrams, J. Pharm. Sci. 94 (2005) 524–539.
[25] S. Šašić, Y. Katsumoto, H. Sato, Y. Ozaki, Applications of moving window two-dimensional correlation spectroscopy to analysis of phase transitions and spectra classification, Anal. Chem. 75 (2003) 4010–4018.
[26] M. Thomas, H.H. Richardson, Two-dimensional FT-IR correlation analysis of the phase transitions in a liquid crystal, 4′-n-octyl-4-cyanobiphenyl (8CB), Vib. Spectrosc. 24 (2000) 137–146.
[27] D. Zhou, G.G.Z. Zhang, D. Law, D.J.W. Grant, E.A. Schmitt, Physical stability of amorphous pharmaceuticals: importance of configurational thermodynamic quantities and molecular mobility, J. Pharm. Sci. 91 (2002) 1863–1872.
[28] E. Joiris, P. Di Martino, C. Berneron, A.-M. Guyot-Hermann, J.C. Guyot, Compression behavior of orthorhombic paracetamol, Pharm. Res. 15 (1998) 1122–1130.
[29] M. Sacchetti, Thermodynamic analysis of DSC data for acetaminophen polymorphs, J. Therm. Anal. Calorim. 63 (2001) 345–350.
[30] G. Perlovich, T. Volkova, A. Bauer-Brandl, Polymorphism of paracetamol. Relative stability of the monoclinic and orthorhombic phase revisited by sublimation and solution calorimetry, J. Therm. Anal. Calorim. 89 (2007) 767–774.