Polymorphism in 2-Chlorobenzamide – Run of the Mill or Not?

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ABSTRACT: Structures of two very similar polymorphic forms of 2-chlorobenzamide have been obtained at low temperature. The metastable α-form is very susceptible to grinding, a property not initially realised considering the polymorphs have been reported in the literature for many years. Systematic milling studies have been carried out in a search for other polymorphic forms and to assess the rate of any polymorphic change(s). No new polymorphs were found, though a computational search for low energy structures suggested a complex crystal-energy landscape. An experimental polymorph screen was also carried out.

Introduction

The molecule 2-chlorobenzamide has two previously published structures[1] that were obtained at ambient temperature and reported in the CSD[2] with reference codes ('refcodes') CLBZAM10 for the α-form (dimer motif) and CLBZAM11 for the β-polymorph (stepped chain motif). The α-form crystallizes in the monoclinic space group $P2_1/n$ whilst the β-form crystallizes in the orthorhombic space group $P2_12_12_1$ with both polymorphs having one molecule in the asymmetric unit, i.e. $Z' = 1$. The earlier reported crystal structure determinations were made using two-dimensional X-ray Weissenberg photography.[1] The unit cell dimensions of the two polymorphs are very similar. According to Kato, Takaki and Sakurai's research,[1,3] two other forms, γ and δ, were obtained 'accidentally' but they failed to reproduce these phases, which were assumed to be disordered or mixed phases closely related to the α- and β-forms. We investigate the polymorphism of 2-chlorobenzamide by carrying out a polymorph screen, and, after finding that the α-form is very sensitive to grinding, carry out a controlled investigation into the grinding.

Infrared spectroscopy (IR) was initially used by us as a monitoring tool to assess polymorphic outcome of crystallization experiments. However, sample preparation involves a sample being subjected to a degree of pressure and grinding so we suggest that the previous reporting of other phases, or mixed phases, may be related to the sensitivity of the α-form. We successfully obtained high-quality low-temperature structures for the α- and β-forms and these were used as the basis for theoretical predictive studies on 2-chlorobenzamide. The sensitivity to grinding was studied systematically using a Retsch mill and searches were made both experimentally and theoretically for the previously proposed intermediate forms or mixed phases.

Benzamide, itself, has two polymorphs with unit cells of very similar size and a largely forgotten metastable phase of transient existence, characterized relatively recently using synchrotron data.[4,5] Furthermore, there were, as with 2-chlorobenzamide, similarities in the packing motifs of the polymorphs.[6] Relatively recent predictive studies on benzamide identified the three polymorphs in a complex energy landscape. The authors[7] suggest that the metastable polymorph described by

Wöhler and Liebig 175 years ago could have been solved earlier, had CSP methods been available. Other benzamides, notably 4-chlorobenzamide, are also known to be polymorphic.[8,9,10]

Experimental

2-Chlorobenzamide, as a white powder, was obtained initially from Sigma-Aldrich (β-form, 98% pure), and latterly Alfa Aesar (α-form, 98% pure) and used without further purification. The underlying habit is always needles, whatever the source and nature of any subsequent crystallization experiment. The onset of melting is 141°C for the α-form and the β-form undergoes a solid-solid phase transformation to α prior to melting (see SI for further details).

Single crystal X-ray diffraction. Crystals were mounted on 20 μm nylon loops from Hampton Research and X-ray diffraction data were recorded on an Agilent SuperNova Dual Source Diffractometer with Cu Kα radiation ($\lambda = 1.5418$ Å) at 150 K. Unit cell determination, data reduction and absorption corrections were carried out using CrysAlisPro (vers. 171.37). The structure was solved by direct methods and refined by full-matrix least-squares on the basis of using SHELX 2013 within the OLEX2 GUI. Non-hydrogen atoms were refined anisotropically and hydrogen atoms were included using a riding model.[11,12,13]

Infrared spectroscopy. Spectra were recorded on a PerkinElmer SpectrumOne™ instrument fitted with a diamond ATR accessory. Spectra were recorded at 4 cm^{-1} resolution over the spectral range 4000-650 cm^{-1}. Samples (typically 1-2 mg) were placed on the surface of the ATR crystal and subjected to the requisite amount of pressure. Care was taken to ensure that samples were subjected to no lateral grinding in the sample preparation phase.

Ball-mill grinding. A Retsch mill, model MM200, was used in controlled and systematic milling studies. Balanced stainless-steel barrels (18 mm Ø × 60 mm) with two 4 mm diameter stainless steel balls (and latterly one ball in an attempt to reduce static electricity in the milled sample) were used at an agitation rate of 25 Hz.

DSC. Measurements were performed on a Q2000 DSC (TA Instruments, LLC, Waters) using Tzero aluminium pans and non-hermetic lids and dry nitrogen as a purge gas at a flow rate of 50 mL/min. Temperature and enthalpy calibration were performed with indium (onset temperature error is +/- 0.5 °C and enthalpy +/- 2%). Samples of an average mass of 1.7 +/- 0.1 mg were heated from 0 to 180 or 250 °C at rates of 10 or 100 °C/min respectively. Data were collected with TA Advantage software (version 5.5.3) while peak integration was performed with the sigmoidal integration function in TA Universal Analysis software (version 4.5A).

Hot-stage microscopy. Hot-stage microscopy was carried out using a Kofler hot-stage microscope (Reichert, Austria).

Manual Polymorph Screening. The commercial material (initially from Sigma-Aldrich) was used in previous crystallization experiments. Several crystallization methodologies were used in the search for novel polymorphs of 2-chlorobenzamide. These included slow evaporation, cooling, vapour diffusion, and liquid diffusion, typically using 10-100 mg of 2-chlorobenzamide dissolved in 1-5 ml (in 5 ml vials) or 5-20 ml (in 25 ml vials) of solvent[15] (See SI for further details). Many crystallization experiments were subsequently repeated when issues associated with grinding of the α-form became apparent.

Results and Discussion

The α-form (xstr0419) has a similar packing to that observed in 2-fluorobenzamide[14] with two types of hydrogen bonds, one giving rise to a dimer and the other providing a link to produce a chain structure. The β-form (xstr0420) displays a stepped motif rather than a dimer (Figure 1).

Both structures are consistent with the previous reports but display much greater precision in the metric parameters.

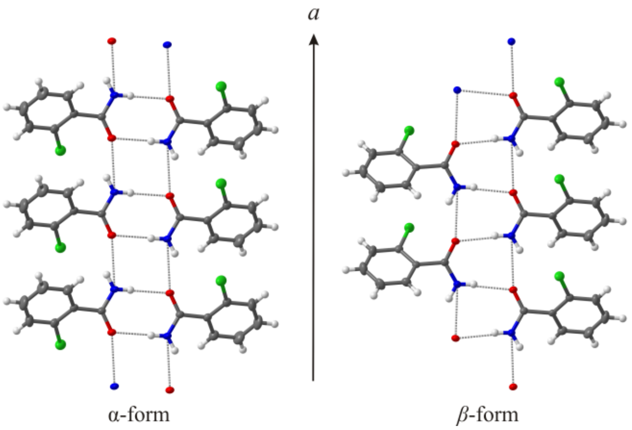

Figure 1. Dimer and stepped motifs associated with the α- and β-polymorphs of 2-chlorobenzamide that run along the *a*-direction of each unit cell. The lattice parameter *a* is approx. independent of temperature for both forms (see **Figure SI 5.2**).

Powder X-ray diffraction was initially used to complement IR characterization methods but as all samples exhibited a needle habit it was felt that grinding and sieving associated with sample preparation for PXRD would likely cause conversion in the case of the α-form, resulting in the formation of mixed phases or even full conversion to the β-form. Attempts were made to grow the two polymorphs on flush silicon wafers but the resulting diffraction patterns exhibited very strong preferred orientation and the process of carrying out polymorph screening in this way and on a large scale was felt to be unrealistic. An additional problem with PXRD studies on 2-chlorobenzamide is that the unit cell parameters for the two forms are very similar (and the monoclinic cell angle for the α-form is very close to 90° at room temperature, see **Figure SI 5.4**) and so the 2θ positions of the diffraction peaks for the two polymorphs are nearly identical.

Infrared spectroscopy was used to quickly and efficiently categorize samples obtained from manual polymorph screening experiments. Infrared uses ATR (Attenuated Total Reflectance) which involves pressure but no lateral grinding of the samples (the cause of polymorphic sensitivity of the α-phase). However, considerable care is still required in the sample preparation stage. A spectrum of the commercial material (from Sigma-Aldrich) was collected and found to correspond to the β-form (**Figure 2**, top) and was subsequently used as a reference spectrum. Crystals grown in acetonitrile, for example, appeared to be of the β-form, bearing a close resemblance to the reference spectrum. In contrast crystals grown in chloroform, dichloromethane, isopropanol and in one case from dioxane, give spectra with key differences notably in the NH (strong peaks at 3365 and 3186 cm^{-1} in β-2-chlorobenzamine shift to 3356 and 3168 cm^{-1} in α-2-chlorobenzamide) and carbonyl stretching regions (peak at 1633 cm^{-1} in the β form shifts to 1648 cm^{-1} in the α form). Full spectra, with all peaks labelled, are given in **Figure SI 1.2**. The crystals that produced the spectrum used as a reference for the α polymorph (**Figure 2**, bottom), obtained from DCM, were confirmed, by single crystal X-ray diffraction, to correspond to the α-form.

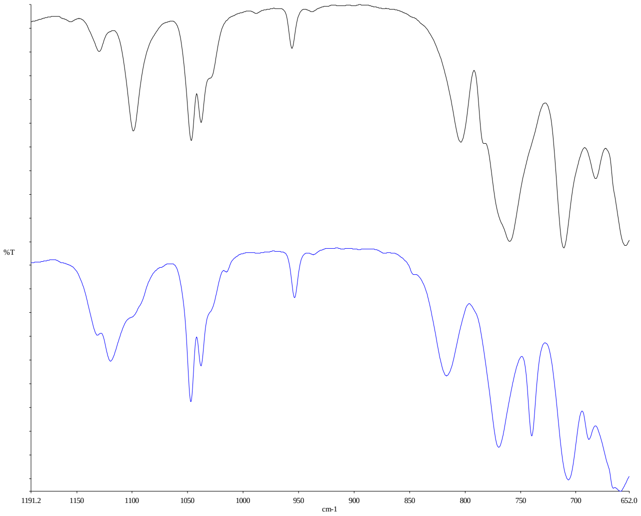

Figure 2. Expanded IR reference spectra (fingerprint region 1190-650 cm^{-1}) of the commercial material supplied by Sigma-Aldrich (top; β-form), material crystallized from DCM (bottom; α-

form). The full IR spectra are available in Supplementary Information - see **Figures SI 1.1 and 1.2**.

The chemical purity of the commercial material was 98% in both cases. LC mass spectroscopy studies did not suggest a different route of manufacture and were consistent with a difference in choice of final recrystallization solvent.

Although many of the infrared spectra of samples resulting from our crystallization experiments broadly corresponded to either the α- or β-form, others appeared to be of an intermediate phase (or phases). In the earlier research associated with 2-chlorobenzamide[1] there was a suspicion of the existence of other polymorphs, possibly other metastable form(s) or a mixture of forms. Whilst the IR spectra when viewed superficially seemed to confirm this observation, spectra obtained on the same batch of the α-form showed differences depending on the level of grinding employed. In the light of this observation a simple but formal grinding experiment was instigated. A sample of α (obtained by recrystallization of Sigma-Aldrich material from chloroform) was milled in a Retsch mill for 5 minutes, 30 minutes and 2 hours, and IR spectra collected (see **Figure SI 1.3**). The NH stretching band at ca. 3350 cm^{-1} shifted to higher wavenumber. The key issue to note is that some effect of grinding is observed after a short period of time (5 mins or less) but up to 2 hours of milling is required to effect complete conversion to the β-form. There were also obvious changes in the complexity and appearance of the carbonyl amide 1 band (ca. 1645 cm^{-1}). It is likely that the infrared spectrum is reflecting mixtures of the α- and β-forms. This is certainly true of spectra obtained before the grinding sensitivity became evident. In some cases (e.g. crystallization from THF, in an early experiment) the high frequency NH band was observed at 3359 cm^{-1}, suggesting that sample preparation may have already contributed to the generation of a mixed phase.

As previously noted, the underlying habit for all samples of 2-chlorobenzamide is needle-like of variable dimensions (some very long filaments, other samples 'cotton-wool' like), dependent primarily on solvent and concentration. The outcome of the milling experiment suggests that even minimal grinding produces signs of changes in the spectral fingerprint of the α-form. Spectra known to be of the β-form overlay very well with each other and are, to all intents and purposes, identical. The definitive NH stretching frequency for the so-called α-form is less well defined and is dependent on crystallization technique and sample preparation, and can differ from batch to batch (two batches of the α-form recrystallized from DCM produced samples where the NH stretching frequency was observed at 3356 cm^{-1} (usual observation) but as low as 3353 cm^{-1} on one occasion). It should be reiterated that minimal milling causes a shift in the NH stretching frequency whilst it takes 2 hours of intense milling to ensure complete conversion of the α-form to the β-polymorph. Further, if mixtures of the α- and β-polymorphs are mathematically co-added using the IR spectrometer software then the resulting spectra appear to be consistent with what initially appeared to be an intermediate phase.

In one case, milling for 2 hours resulted in the production of what appeared to be an amorphous sample of 2-chlorobenzamide, which was assessed by a lack of birefringence under polarized light microscopy. This observation could not be reproduced. The 'amorphous' material readily recrystallized to the β-form by liquid assisted grinding for 2 minutes in the Retsch mill. Whilst the α-form is very sensitive to grinding when recrystallized from the β-form, it is relatively stable to a downward pressure. However, the needles produced are so fragile that it is difficult not to produce any conversion when manipulating samples of the α-form.

DSC confirmed the melting point of 141 °C for the α-form. An apparent solid-solid phase transformation was associated with both forms, onset 114 °C for the α-form and 131 °C for the β-form, the latter being conversion to the α-form (**Figures SI 2.1, 2.2)**. These pre-melting events were further examined using hot-stage microscopy. In the case of the α-form there was a *minimal* degree of melting just prior to long sublimed needles developing below 120 °C, which were confirmed by single crystal X-ray diffraction to be the α-form. Heating the β-form at a higher rate shown in **Figure SI 2.3 s**uggests that the pre-melting transition was inhibited. Subsequently, a variable temperature PXRD study on the commercial materials showed no phase transition for the α-form on heating (other than melting), but a clear phase transition to the α-form from the β-form at approx. 135 °C (**Figure SI 5.1**).

IR examination following recrystallization from the melts of both the α- and β-forms confirmed the polymorphic outcome to be the α-form in both cases. It was not possible to calculate the enthalpy of fusion for either of the forms due to the pre-melting transition; however, the conversion of the β-form to the α-form suggests an enantiotropic relationship. This could mean that the α-form is stable at higher temperature while the β-form is stable at room temperature, which would explain the conversion of the α-form to β-form on milling at room temperature. The PXRD study of lattice parameters and unit cell volume change as a function of temperature (**Tables SI 2.1, 2.2 and Figures SI 5.2-5.4**) suggests that the β-form should always be more stable in the absence of thermal entropic effects, given that it has a denser structure (and other factors being equal) over the whole temperature range. (Heating the β-form at a higher rate as shown in **Figure SI 2.3** suggests that the pre-melting transition is inhibited.)

A CrystalPredictor search was successful in finding the β-form of 2-chlorobenzamide. Following CrystalOptimizer refinement, structures that matched both forms were found. Although the structures that matched the experimentally observed forms were not the lowest in energy on the crystal energy landscape, they were very close in energy to one another. All the structures were compared with the experimentally observed forms. The settings used in XPac allowed structures whose conformation had the amide group rotated by 180° to be matched as the same. Full analysis of the structures is given in the SI. Most of the low energy structures depicted in **Figure SI 3** have similarities with the experimental structures. The landscape appears to fall into two parts. The double chains, Chains 1-4, **(see SI)** are various ways of doubling the Chain 5 unit that is the common building block of the two experimentally observed forms. This unit is seen in almost all the lower density group of structures observed on the crystal energy landscape. The band of structures with slightly higher density and energy mainly show only dimer interactions in common with either of the experimental structures, with all other interactions being non hydrogen bonding van der Waals interactions. Hence, the landscape is separated into the lower energy, lower density hydrogen bonded structures and the higher energy but denser van der Waals structures.

The energy landscape shows that there are different ways of packing the chains that are common to the two structures. Given this energy landscape and no experimental knowledge of the system, a computational chemist might postulate that there are a number of polymorphs, or a disordered system prevailing. Experimentally only two closely related polymorphs (see **Figure SI 3**) were found despite extensive polymorph screening.

Given the similarity of the structures of the two polymorphs, the sensitivity of the α-form to grinding and the hint of 'intermediate' phases, this molecule was chosen as a model to be studied on the Long Duration Experiment (LDE) initiative at the Diamond Light Source. The ease of conversion of the α-form to the β-form also appeared to be related to storage conditions (temperature and humidity). The prototype experimental setup in the LDE takes account of the sensitivity of the α-form to grinding and milling and minimal sample preparation is employed.

Conclusions

We have redetermined the X-ray crystal structures of the α and β forms of 2-chlorobenzamide at low temperature on modern X-ray diffractometer. Based on our work to date there is no tangible evidence for defined intermediate phases previously alluded to. The high quality structures obtained during the course of this work show no evidence of disorder associated with them. Given the complex energy landscape from CSP, the potential for mixed phases is likely, possibly involving variation in chain stacking / faults. It is hoped that the LDE may shed light on this.

ASSOCIATED CONTENT

Supporting Information. Details of the polymorph screen of 2-chlorbenzamide; expanded IR spectra; DSC data; the computed crystal energy landscape; calculated powder patterns from single crystal X-ray structures; Results of variable temperature PXRD experiments. This material is available free of charge via the Internet at http://pubs.acs.org

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

EPSRC grants EP/K039229/1 “Computationally Designed Templates For Exquisite Control Of Polymorphic Form” and EP/K03930X/1 “Core Capability for Chemistry Research - University College London” are acknowledged for supporting this work.

REFERENCES

1. Kato, Y.; Takaki, Y.; Sakurai, K. *Acta Crystallogr., Sect B.* 1974, *30*, 2683-2687.
2. Allen, F. H. *Acta Crystallogr., Sect B.* 2002, *58*, 380-388.
3. Takaki, S.; Kato, Y.; Sakurai, K. *Acta Crystallogr., Sect B.* 1975, *31*, 2753-2758.
4. David, W. I. F.; Shankland, K.; Pulham, C. R.; Blagden, N.; Davey, P. R. J.; Song, M. *Angew. Chem. Int.Ed.* 2005, *44*, 7032-7035.
5. Blagden, N.; Davey, R.; Dent, G.; Song, M.; David, W. I. F.; Pulham, C. R.; Shankland, K. *Cryst. Growth Des.* 2005, *5*, 2218-2224.
6. Thun, J.; Seyfarth, L.; Butterhof, C.; Senker, J.; Dinnebier, R. E.; Breu, J. *Cryst. Growth Des.* 2009, *9*, 2435-2441.
7. Thun, J.; Schoeffel, M.; Breu, J. *Molecular Simulation* 2008, *34*, 1359-1370.
8. Takaki, Y.; Nakata, K.; Taniguchi, T.; Sakurai, K. *Acta Crystallogr., Sect B.* 1978, *34*, 2579-2586.
9. Taniguchi, T.; Nakata, K.; Takaki, Y.; Sakurai, K. *Acta Crystallogr., Sect B.* 1978, *34*, 2574-2578.
10. Hayashi, T.; Nakata, K.; Takaki, Y.; Sakurai, K. *Bull. Chem. Soc. Jpn.* 1980, *53*, 801-802.
11. CrysAlisPro, vers. 171.37, Agilent Technologies UK Ltd, Yarnton, England (now Rigaku Oxford Diffraction®).
12. Sheldrick, G. M. *Acta Crystallogr., Sect A.* 2008, *64*, 112-122.

13. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *J. Appl. Cryst.*, 2009, *42*, 339-341.

14. Kato, Y.; Sakurai, K. *Bull. Chem. Soc. Jpn.* 1982, *55*, 1643-1644.

15. Ntantou, A. MSc thesis, University College London, 2007.

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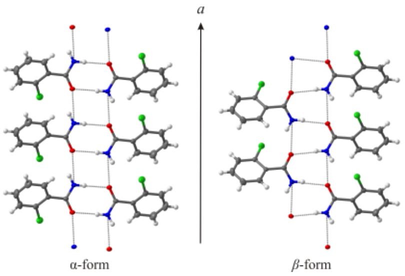

We have redetermined the crystal structures of the α and β forms of 2-chlorobenzamide. Following an extensive polymorph screen, the susceptibility of the α form to grinding has been investigated, and there is no evidence for the previously alluded to intermediate phases or other phases.