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Polymorphism

Polymorphism in Benzamide: Solving a 175-Year-Old Riddle

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One hundred seventy-five years ago, Friedrich Wöhler and Justus von Liebig described the first incidence of polymorphism with a molecular crystal.[1] Today thermodynamic and kinetic factors associated with polymorphism still represent major challenges in solid-state research. Despite the pronounced impact of polymorphism[2] on physical and chemical properties (e.g. solubility, shelf life) and the legal and economic implications resulting from these differences (see the case of Ritonavir[3,4]), the deliberate and reproducible synthesis of metastable polymorphs from solution remains difficult and is far from trivial. It entails controlling the nucleation of the desired form as well as simultaneously suppressing nucleation of competing congeners. If secondary nucleation cannot be prevented, the metastable form will be transformed by dissolution and reprecipitation into the more stable form. The difference in lattice energy and hence solubility is the driving force for the transformation. Large differences will render metastable forms transient and difficult to capture.[5]

By chance, the very first known molecular dimorph, benzamide, appeared to form crystals with metastable modifications. Wöhler and Liebig described that slow cooling of a “boiling hot” aqueous solution of benzamide resulted in a white mass of silky needles. After a few hours or days they observed a phase transformation in solution towards well-formed rhombic crystals. This stable monoclinic form (form I) was thoroughly investigated, and the crystal structure was solved in 1959 by single-crystal X-ray diffraction.[6] The metastable form was forgotten for over 150 years, and the existence of this second phase was referred to only in passing.[7] No crystal structure was available—a rather ominous fact for a simple molecule with limited torsional flexibility. In 2005 David et al. solved the structure of a new metastable polymorph (form II) from X-ray powder diffraction data[8] that were rapidly recorded (< 60 min.) at a synchrotron source. The crystals of form II were obtained in situ on the diffractometer by cooling a dilute solution (0.173 M) in a flame-sealed glass capillary (1.5 mm). Initially the solution was cooled to 2 °C followed by flash cooling “through brief contact with a cotton bud soaked in liquid nitrogen”. In this way the relative proportion of form II over form I could be increased to a maximum of 17.3 %. Please note that these crystallization conditions deviate dramatically from those in the original experiment described by Wöhler and Liebig. In a subsequent full paper Bladgen et al. showed that the solid-state transformation of the highly metastable form II into form I is facile and complete within 3 h.[9]

We repeated the original experiment in an automated lab reactor (LabMax, Mettler Toledo) equipped with online sensors that afford more controlled crystallization conditions. The saturation level was monitored with an ATR FT-IR probe (ReactIR, Mettler Toledo), and a FBRM probe (focused beam reflectance measurement, Lasentech) detected nucleation almost in real time. In order to replicate Wöhler’s experiment a concentrated hot aqueous solution of benzamide (0.347 M) was cooled with cooling rates ranging from 0.1 $\mathrm{K\,min^{-1}}$ to 5 $\mathrm{K\,min^{-1}}$. As soon as crystals were detected by the FBRM probe and/or a decrease in the supersaturation was detected, the suspension was filtered and the crystals were dried. Using very low cooling rates only form I could be found; there was no sign of any other form. However, with cooling rates of about 3 $\mathrm{K\,min^{-1}}$ additional reflections appeared in the diffractogram that did not belong to either form I or form II. A mixture of this new phase and the known form I could be synthesized by sublimation as well. Form II was not observed in any of these experiments.

X-ray powder diffraction (XRPD) patterns recorded with $\mathrm{Cu_{K\alpha1}}$ radiation and a modern high-speed/high-resolution detector[10] allowed the identification of 13 diffraction peak positions that are attributable to a new form (form III). These could be indexed by applying the program DICVOL91[11] to give a monoclinic unit cell ($a = 22.5010$, $b = 5.5159$, $c = 5.0569$ Å, $\beta = 91.36$ °) with a good de Wolff figure of merit (FOM = 29.4). The dimensions of the unit cell is very similar to that of form I ($a = 5.5657(9)$, $b = 5.0353(9)$, $c = 21.698(4)$ Å, $\beta = 90.388(3)°$),[12] and peak overlap is severe (Figure 1). Additionally, the peaks unique to form III happen to be rather weak. Therefore, when low-resolution and low-intensity diffraction data are used, form III can be overlooked easily. The unit cell volume is 626.68 Å^3, which is slightly more than that of form I (608.1(2) Å^3). Following a Pawley refinement[13] the possible space groups were identified to be either Pc or $P2_1/c$. The centrosymmetric space group $P2_1/c$ was chosen as the most likely candidate for the crystal structure. The structure solution was performed in real space by global optimization methods (simulated annealing with metropolis algorithm) applying PowderSolve.[14] The molecular geometry of the benzamide molecule (including all torsions) was taken from the known monoclinic crystal structure. Several independent simulated annealing runs were conducted, and the same minimum was found in all runs.

Unsurprisingly, a rather bad R_{wp} value of 23 % was obtained in the solution runs due to the fact that the global

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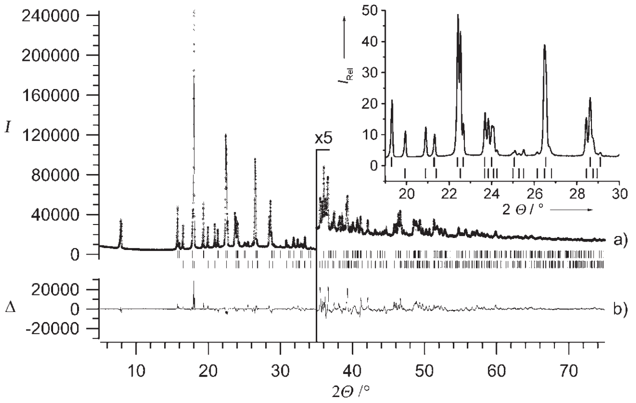

Figure 1. Scattered X-ray intensities as a function of diffraction angle showing the good fit between the observed and calculated data based upon a two-phase refinement. Shown are a) the observed pattern (◇), the best Rietveld-fit profile (——), and b) the difference curve between observed and calculated profiles. Intensities of the high-angle part are enlarged by a factor of 5. The insert underlines the severe overlap of form I and form III peaks. The upper and the lower tick marks indicate the positions of the Bragg reflections of form I and form III, respectively. I = intensity; I_{Rel} = relative intensity.

optimization runs were performed with a diffraction pattern consisting of contributions of two phases. The very severe overlap, evident in the insert of Figure 1, cannot be handled in the PowderSolve module. Finally, a high-resolution X-ray powder diffraction pattern of benzamide was recorded in transmission at room temperature on a laboratory powder diffractometer.[15] The sample was spun during measurement for better particle statistics. The final Rietveld refinement of the crystal structure was performed with the program TOPAS[16] (refinement details are given in Table 1). Both phases were refined simultaneously with each independent benzamide molecule modeled by using a Z-matrix description. All torsions were refined as well, and separate isotropic temperature factors for each phase were required to obtain an adequate structural refinement.

The final fit between the observed and calculated diffraction patterns is shown in Figure 1, and the figures of merit (see Table 1) confirm the good fit. Modeling a disorder of the amide group did not yield a significantly better profile fit; the existing difference between the calculated and observed diffraction patterns might be attributed to stacking faults or other kinds of disorder in the new form III. The sample was found to be a mixture of 1:1 ratio of the monoclinic forms I and III.

Table 1: Rietveld refinement details for the laboratory XRPD data obtained at room temperature.

Parameter	Mixed-phase refinement	
	Form I	Form III
a [Å]	5.6094(1)	5.0551(1)
b [Å]	5.0399(1)	5.5141(1)
c [Å]	22.1171(8)	22.9565(9)
β [°]	90.641(2)	101.292(2)
V [Å^3]	625.23(3)	627.50(3)
space group	$P2_1/c$	$P2_1/c$
Z	4	4
Z'	1	1
phase fraction	51.6(4)	48.4(4)
data range 2Θ [°]	5–75	
R_{Bragg}	5.33	5.32
R_{wp}	6.95	
zero point	−0.0116	

Additionally, a ^{13}C CP-MAS solid-state NMR spectrum was recorded at ambient temperature (Avance II 300, Bruker). The data collection was performed applying broadband proton decoupling using a SPINAL-64 sequence.[17] Deconvolution of the resonance at about $\delta = 173$ ppm clearly reveals two independent carbonyl signals in agreement with expectations based on XRPD results (Figure 2). Both, form I and form III, contain one molecule in the asymmetric unit, and hence two independent carbonyl signals in the ^{13}C solid-state NMR spectra are expected for the concomitantly crystallizing mixture of the two phases.

It should be noted that XRPD and solid-state NMR experiments were both performed several weeks after crystallization. Periodic checks with XRPD indeed gave no hints even for a slow solid-state conversion of form III into form I.

The most important intermolecular interaction for benzamide is of course hydrogen bonding. Unsurprisingly, the hydrogen bonding motif is therefore very similar in all three solid phases. There are minor differences resulting from different torsion angles, but all phases show hydrogen-bonded dimers connected by further hydrogen bonds to form long ribbons along the short axis of the unit cell. The significant differences in the observed packing motifs are, however, triggered by the second-ranking intermolecular interaction, the π–π interaction of the benzyl moieties. The favored π–π interaction motifs are shifted π stacks and T-shaped interactions resulting in herringbone-type motifs. And indeed, great differences in the π–π stacking motifs are observed (Figure 3). Form I is dominated by shifted π stacks. In both metastable forms herringbone patterns are observed. In Form II pairs of hydrogen-bonded ribbons have a T-shaped

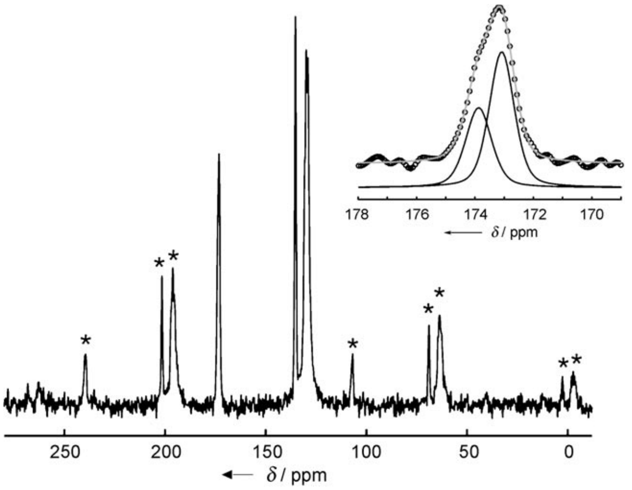

Figure 2. ^{13}C CP-MAS spectrum (ν_{rot} = 5 kHz) of a mixture of form I and form III. Spinning sidebands are marked with asterisks. The insert shows the carbonyl signals with the corresponding fit (gray line) and the deconvoluted resonances (black lines). The two clearly visible independent resonances represent the two different phases of benzamide.

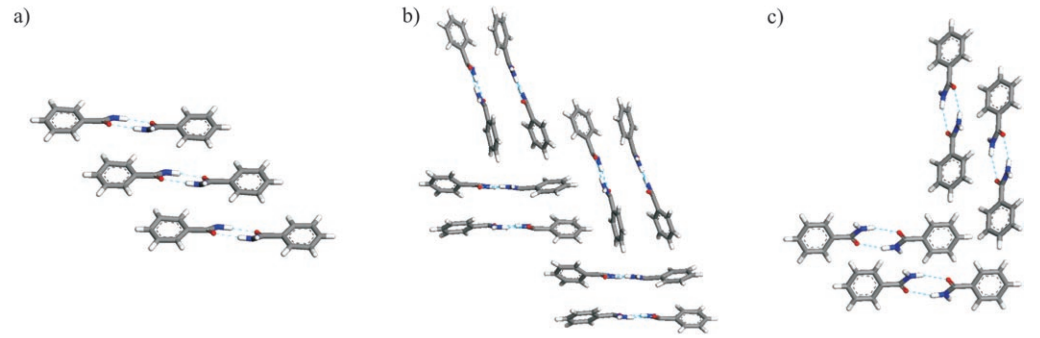

Figure 3. A projection along the ribbon axes shows the π–π interactions in the different forms. a) Form I, view along [010]; b) form II, view along [001]; c) form III, view along [100].

arrangement, while in the new form III the herringbone pattern consists of single ribbons.

Thus the π–π interactions seem to be the major difference in three crystal forms of benzamide. Lattice energy calculations using intermolecular potentials from the Dreiding force field in combination with partial charges derived from the molecular electrostatic potential (MEP)[18] confirmed the observed relative stabilities of the different phases. Lattice energies of the minimized structures are listed in Table 2. The energetically most favorable hydrogen-bonding motif[20] is found with the thermodynamically stable form I, but the best π–π stacking motif is realized in the new form III. The rather pronounced differences in lattice energies might indicate why form II is rapidly converted into form I, while form III seems to be rather persistent.

Table 2: Lattice energy calculations with the Dreiding force field, computed with MS Modeling 4.0.[22]

U_{Latt}[a] [kcal mol^{-1}]	Form I	Form II	Form III
total[b]	−28.06	−22.75	−25.47
van der Waals[b]	−11.06	−9.05	−11.19
coulombic[b]	−10.25	−10.12	−11.00
hydrogen bond[b]	−6.75	−3.58	−3.28

[a] Lattice energy per molecule. [b] Without intramolecular interactions.

175 Years after the first report of polymorphism in benzamide a second metastable phase has been characterized. Since this new phase was obtained under conditions closely resembling those of the original experiment, although more controlled, this form III most likely represents the silky needles described by Wöhler and Liebig. Form III is metastable but persistent as a dry solid; it can easily be produced in every crystallization run following Wöhler's and Liebig's cooling rates. While form II is a transient metastable structure and escaped characterization by "running away", form III simply managed to "hide" behind the stable form I for such a long time. Crystallization techniques taking advantage of modern online sensors like FBRM probe and ATR FT-IR technology helped to increase the relative proportion of the metastable form III above the detection limit. However, so far we failed to completely eliminate the thermodynamically stable form I.

Lattice energy calculations are perfectly in line with Ostwald's rule. Flash cooling as applied by David et al. generates the highest supersaturation and leads to the most instable polymorph. Medium supersaturations caused by cooling rates as applied by Wöhler and Liebig yield form III which is only slightly less stable than the thermodynamically stable form I.

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