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Iterative High-Throughput Polymorphism Studies on Acetaminophen and an Experimentally Derived Structure for Form III

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The discovery of crystal polymorphs is often a costly and time-consuming process.[3] There are no failsafe methods to predict the extent of polymorphism of a given compound.[4] We have developed a system, CrystalMax, for high-throughput, parallel polymorph discovery capable of eliciting both stable and metastable forms. It has three main components: experimental design and execution software, robotic dispensing and handling hardware, and high-speed microanalytics. This system was used to assess the extent and nature of polymorphism in acetaminophen (paracetamol; *p*-hydroxyacetanilide), a widely used NSAID.[5] Three polymorphs are experimentally known,[6] one of which (form III) has only been found by thermal microscopy.[7] We report a series of iterative high-throughput experiments for generation and identification of acetaminophen polymorphs. In addition, a structural suggestion for form III is advanced on the basis of powder X-ray microdiffraction (PXRD) and prior computer predictions.

Scheme 1 provides an overview of the CrystalMax system, which facilitates parallel screening of thousands of crystallization conditions. Experimental design software defines combinatorial test conditions on the basis of the selection of solvent properties and methods used to drive supersaturation.[8] Experiments are executed in arrays of individually addressable sample containers.[9] Samples that crystallize are removed from the original array, the solvent is removed by aspiration, and the residue is dried. Optical imaging and in situ Raman spectroscopy are used to characterize newly formed crystals.[10] Polymorph assignments are confirmed by PXRD, DSC, TGA, and optical microscopy. The experiment is continuously tracked by a database system. Cheminformatics software aids automated data analysis and experiment design.

A classification process aids the analysis of the large number of Raman spectra generated during an experiment. Similarity coefficients[11] are calculated for all pairs of spectra. The coefficients are sorted, color-mapped, and displayed as an *n*-by-*n* matrix for easy visualization. Figure 1 shows a representative Tanimoto matrix generated for 120 Raman spectra of acetaminophen in which all three polymorphs are present.[12] The plot in Figure 1 illustrates that the Tanimoto matrix derived from spectral data is a simple visual way of differentiating polymorphs of acetaminophen.

The process in Scheme 1 and the Tanimoto matrix analysis exemplified in Figure 1 were used iteratively to identify all three polymorphs of acetaminophen. At the end of the first iteration, precipitates had been observed in 9.3% (723) of 7776 crystallization trials, 29 of which were II and the remainder form I.[13] No form III or other metastable forms were identified.[4a] The solids comprised single crystals or powders.

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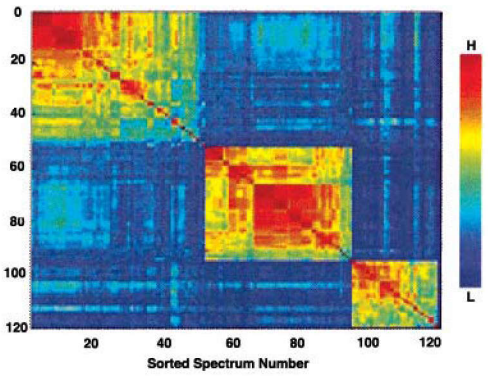
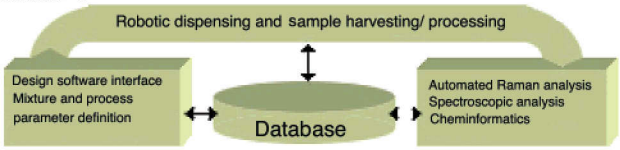


Figure 1. A representative Tanimoto matrix comparing 120 Raman spectra of acetaminophen polymorphs. Each pixel represents the Tanimoto value[11] for pairs of spectra, according to the color scheme on the right. The map displays Tanimoto values for 50, 45, and 25 spectra of forms I, II, and III, respectively. A dark red pixel indicates the highest degree of similarity (Tanimoto ≈ 0.0), whereas dark blue indicates dissimilarity (Tanimoto ≈ 1.0).

Scheme 1



In the second iteration the conditions that gave form II in the initial screen were replicated in higher numbers and showed that only one solvent mixture, 67/33 (v/v) MeOH/toluene, consistently yielded form II.[14] The experiment highlighted the sometimes intractable nature of crystallization in that not all replicates produced crystals and that different polymorphs were obtained from seemingly identical conditions. The third iteration was designed to test the reliability of form II crystallization from 67/33 (v/v) MeOH/toluene by isothermal evaporation at 40, 54, and 65 °C.[15,16] These experiments gave primarily forms I and II.[17] A few samples were characterized as glassy solids at the highest temperature. The largest proportion of form II was obtained at 54 °C, for which the Tanimoto matrix analysis showed that 43% of the samples were form II. Form III was not observed in the evaporative crystallizations.[4a]

Melt crystallization in crystallization containers generated form III in several instances.[18] We observed that samples of form III obtained in this format converted to form II within hours. The results from melt crystallization show that the confinement of the acetaminophen melt between microscope slides is not a strict requirement for the formation of form III.[4a]

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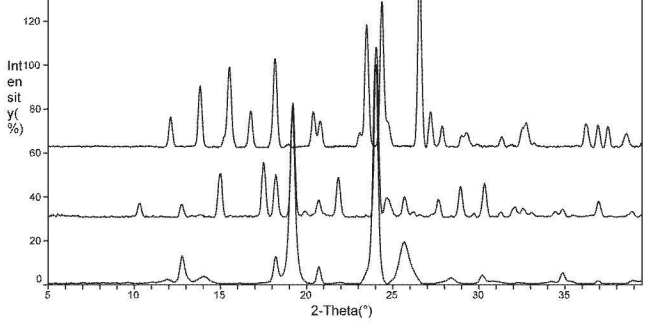

Figure 2. PXRD patterns for forms I (top), II (middle), and III (bottom) of acetaminophen.

We were able to prepare form III in capillaries for PXRD analysis.[19] The diffraction patterns of forms I−III are shown in Figure 2. It is apparent that many of the diffraction peaks of form III are similar to those of form II. This, however, is deceiving and results from accidental overlap of the diffraction peaks of structurally different polymorphs.

We compared the PXRD pattern of form III to those calculated from the known structures of forms I and II and the theoretical structures recently published.[4a] The calculations accounted for crystallite size-dependent line broadening and global isotropic temperature factors.[20] Of the theoretical structures, a monoclinic structure (AK6, $P2_1/c$)[21] gave a calculated powder pattern that closely matched the observed powder pattern for form III. The intensity and width of the low angle peak near 5° 2Θ was found to be very sensitive to the line broadening in the *a*-direction. The apparent absence of this peak in the experimental powder pattern suggests that there is only short-range order along the *a*-axis, which is also the slow growth direction in the calculated morphology.[4a]

The proposed structure is built up of bilayers. Each bilayer is held together by O−H···O(H) hydrogen bonds in the *bc* plane. This hydrogen bonding pattern is strikingly different from that observed in forms I and II where O−H···O(C) and N−H···O(H) hydrogen bonds are observed.[6] Interbilayer interactions are between acetamide methyl groups along the *a*-axis. The weak nature of methyl−methyl contacts is a likely reason for the difficulty in obtaining macroscopic crystals of form III from solution. These weak interactions also facilitate microtwinning along the *a*-axis accounting for the crystallite size-dependent line broadening suggested by the experimental and calculated PXRD patterns.

The CrystalMax platform was used to rapidly prepare and identify three forms of acetaminophen. The benefit of carrying out a multitude of experiments under different as well as identical conditions to capture all metastable forms is evident. A structural model for the elusive third form has been proposed. Future reports on high-throughput crystallization and solid form discovery will focus on understanding how molecular interactions drive crystallization processes and their outcomes.[22]

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Supporting Information Available: Solvents, thermal parameters, and concentrations used for the high-throughput crystallization experi-

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(8) Proprietary Java program, see Supporting Information.
(9) The compound and solvent(s) are placed in sealable glass tubes. In the case of solvent crystallization, supersaturation is generated by thermal cycling the tubes in aluminum blocks. Crystallization events are identified by an optical scanning station using automated image analysis.
(10) Raman spectroscopy is a suitable primary screening method for polymorphs because of its sensitivity to changes in crystal form. In addition, spectra can be obtained from microgram amounts of material in a matter of seconds using standard equipment. See: Bugay, D. E. *Adv. Drug Delivery Rev.* **2001**, *48*, 43−65.
(11) Tanimoto coefficient. Tm = 1 − [Nab/(Na + Nb − Nab)], where Nab is the number of peaks coincident within a user defined range in both spectra a and b, Na is the number of peaks in spectrum a, and Nb is the number of peaks in spectrum b.
(12) The spectra were obtained from a combination of experimental crystallization and authentic materials (the latter verified by melting point, optical microscopy, and/or powder X-ray diffraction).
(13) We conducted thermally driven solution crystallization in 7776 samples representing 2592 unique conditions. Each condition was run in triplicate to test reproducibility of crystallization and to increase the chances of identifying metastable forms. A diverse set of 24 solvents was used, either as single, binary, or ternary solvent mixtures. Crystallization solvents featured a broad range of chemical functionality (complete listing in Supporting Information). Three nominal drug concentrations were employed with each combinatorial solvent condition in an effort to cover a wide range of supersaturation levels.
(14) Each solvent mixture that gave at least one form II crystal was replicated eight times in the follow-up experiment.
(15) Solutions of acetaminophen were prepared in arrays of 96 sealed crystallization containers. The arrays were heated to the desired evaporation temperature, and the seals were removed. The samples were allowed to evaporate and were visually monitored for crystallization, solids were rearrayed, and the crystals were analyzed by Raman spectroscopy. Experiments took 24 h.
(16) The temperatures used are just above the glass transition temperature, the temperature typically used in thermal microscopy experiments to prepare form III and just below the temperature where form III converts to form II, respectively.
(17) A few of the samples prepared at 65 °C were identified as glassy acetaminophen by visual inspection and Raman spectroscopy.
(18) The experiment was carried out at 54 °C with various nucleating surfaces added into the tubes. Crystallization was observed over 24 h. The Tc matrix indicated that out of 96 acetaminophen samples, two were a noncrystalline glass (verified by optical analysis), five were form III, and the remainder were form II.
(19) Microscopy and Raman are consistent with this assignment.
(20) Detailed information is given in the Supporting Information.
(21) Cell constants, nonstandard setting: $a = 16.05$ Å, $b = 5.07$ Å, $c = 9.65$ Å, $\beta = 79.1°$.
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