Drug Crystal Growth

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Selective Crystal Growth of the Anhydrous and Monohydrate Forms of Theophylline on Self-Assembled Monolayers

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Polymorphs and solvates of pharmaceuticals frequently exhibit differences in physicochemical properties such as bioavailability, comminution, and shelf life.[1] The control of polymorphism[2] and pseudopolymorphism[3] is, therefore, critical to pharmaceutical formulation and distribution.[4] Classical methods for the screening and selective growth of polymorphs or pseudopolymorphs involve altering conditions such as the solvent, temperature, and rate of evaporation.[5] Crystallization, however, is often mediated by heterogeneous nucleation, which can occur on the surfaces of dust particles, glass vials, or other foreign templates.[6] It has long been held that crystal growth can be influenced by the structure of the nucleating surface.[7] Accordingly, Langmuir monolayers,[8] thiol self-assembled monolayers (SAMs),[9] silane monolayers,[10] single-crystal substrates,[11] and polymer particles[12] have been used in the growth of organic and inorganic crystals.[13] Herein, we report the effect of the hydrogen-bonding ability of thiol SAMs on the crystal growth of the anhydrous and monohydrate forms of theophylline,[14] a drug widely used as a bronchodilator in asthma therapy. We show that the disparity in the number of hydrogen-bond donors and acceptors in theophylline and the geometric complementarity at the growth interface are critical to the observed selectivity in the crystal growth.

The monohydrate (space group $P2_1/n$) and anhydrous (space group $Pna2_1$) forms of theophylline crystallize concomitantly[15] from ethanol at 70% relative humidity.[16] Figures 1 and 2 show the calculated and experimental morphologies of the two forms. The thiol SAMs used in this work were fabricated on gold-coated glass slides by immersing the slides in ethanol solutions of ω-functionalized alkanethiols.[17] Hydrophobic SAMs were made from 1-dodecanethiol (**1**) and 1-hexadecanethiol (**2**), and hydrophilic SAMs from 11-mercaptoundecanol (**3**), 11-mercaptoundecanoic acid (**4**), and 16-mercaptohexadecanoic acid (**5**).

Crystallizations were carried out in ethanol solution at 20 °C and approximately 70% relative humidity.[18] A nearly saturated ethanol solution of theophylline was heated at 60 °C for 30 min to ensure complete dissolution. After cooling, the solution was transferred to vials containing SAM substrates and allowed to evaporate to yield crystals. All the experiments were performed at least five times, and the results from the repeated trials were qualitatively similar.

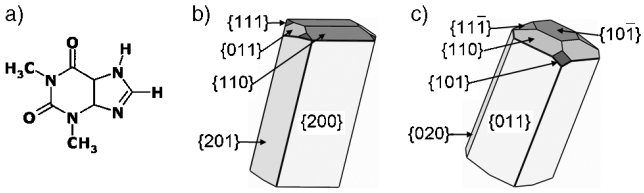

Figure 1. a) The chemical structure of theophylline. Morphologies of b) the anhydrous form and c) the monohydrate form of theophylline calculated using the Bravais–Friedel–Donnay–Harker (BFDH) theory. Symmetry-independent faces are labeled.

Substrates bearing hydrophilic SAM-**3**, SAM-**4**, or SAM-**5** selectively nucleated the anhydrous form of theophylline. Figure 2a shows the crystal growth on carboxy-terminated SAM-**4**. We found that crystals of the monohydrate form of theophylline grew on the inner surfaces of the glass vial containing SAM-**4**. Similar results were obtained with SAM-**3** and SAM-**5**. In the case of SAM-**1** and SAM-**2**, crystals of the monohydrate grew on the SAMs, as well as on the surfaces of the glass vials. We note that the conditions under which the crystal-growth experiments were performed were indistinguishable, save for the differences in the SAMs. These results suggest that SAMs with exposed hydrogen-bonding functional groups act as templates for the selective growth of anhydrous theophylline. In the absence of a SAM, under similar conditions, the crystallizations yielded both the monohydrate and the anhydrous forms, with the monohydrate being the predominant form.

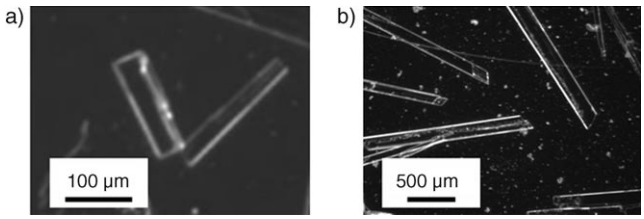

Figure 2. Optical micrographs showing a) the selective growth of the anhydrous form of theophylline on carboxy-terminated SAM-**4**, and b) the growth of the monohydrate form on methyl-terminated SAM-**2**.

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Supporting information for this article (characterization of the SAMs by contact-angle goniometry, ellipsometry, grazing-angle IR spectroscopy, and cyclic voltammetry; characterization of the crystalline forms of theophylline by ATR-IR spectroscopy, thermal methods (DSC and TGA), and powder X-ray diffraction; structural and epitaxial analysis) is available on the WWW under http://www.angewandte.org or from the author.

Noting the influence of hydroxy and carboxy groups on the crystal growth, we evaluated the hydrogen-bonding characteristics of theophylline in both crystalline forms. Theophylline has two acidic hydrogen-bond donors (the imidazole NH and =CH groups) and three acceptors (the two-coordinate imidazole nitrogen atom and the two carbonyl oxygen atoms). Given that each carbonyl oxygen atom can act as an acceptor to two hydrogen bonds, there is a shortage of donors in the theophylline molecule. In the anhydrous crystalline form, the two donors interact with two acceptors, forming hydrogen-bonded bilayers (Figure 3). These bilayers

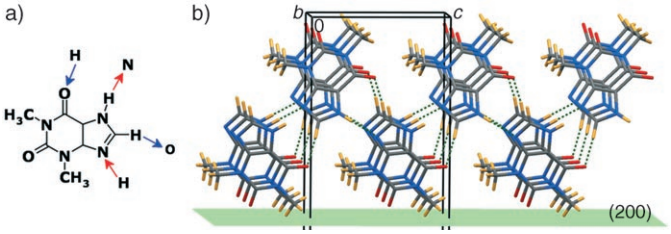

Figure 3. The crystal structure of the anhydrous form of theophylline.[16] a) The hydrogen bonds around a single molecule; the red and blue arrows indicate two distinct hydrogen bonds. b) A molecular bilayer parallel to the *bc* plane; C gray, H yellow, N blue, O red; the green dotted lines indicate hydrogen bonds. Note the presence of exposed non-hydrogen-bonded carbonyl groups at the (200) plane.

are parallel to *bc* plane and expose the third acceptor at (200). Figure 1 b shows that the {200} faces are among the largest on the crystals, and Figure 2 a shows that the crystals lie on the {200} faces on the SAM substrates. We surmised that hydrogen-bond donors, such as hydroxy and carboxy groups on SAMs, interact with the carbonyl acceptors exposed at the {200} faces of the crystal nuclei. These interactions lower the nucleation barrier and promote the selective crystallization of the anhydrous form, though it is thermodynamically less stable than the monohydrate form under the experimental conditions.[16] Powder X-ray diffraction analysis of the crystals showed that the intensities of the (200) and related higher-index reflections are increased compared to those of other reflections (Figure 4), indicating that SAM-**3**, SAM-**4**, and SAM-**5** nucleated the {200} faces of the anhydrous form.

To test whether the geometry (that is, the two-dimensional periodic structure) of the SAMs played any role in the observed selectivity in the crystal growth, we performed crystallizations of theophylline (under similar experimental conditions) using glass slides with exposed hydroxy groups.[19] Unlike hydrogen-bonding SAM-**3**, SAM-**4**, and SAM-**5**, the hydrophilic glass slides consistently yielded a concomitant mixture of the anhydrous and monohydrate crystalline forms. The hydroxy groups on the glass slides promoted the growth of the anhydrous form, but not as exclusively as SAM-**3**, SAM-**4**, and SAM-**5**. These results show that the geometry of the thiol SAMs enhances the selective growth of anhydrous form of theophylline.

We evaluated the geometric complementarity between the SAMs and the crystal faces of both crystalline forms of theophylline using the lattice-matching program EpiCalc.[20]

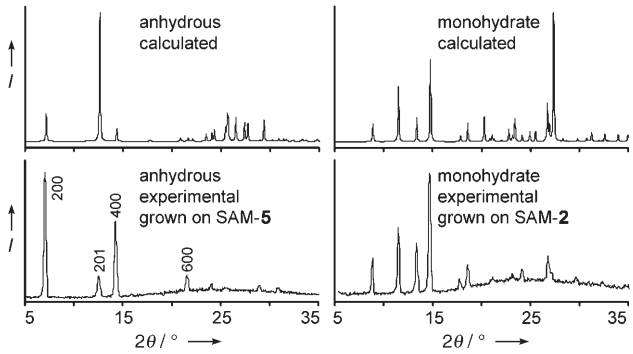

Figure 4. Top: powder X-ray diffraction patterns of the anhydrous (left) and monohydrate (right) forms of theophylline calculated from their crystal structures.[16] Bottom: experimental powder X-ray diffraction patterns of the anhydrous form grown on SAM-**5** (left) and the monohydrate form grown on SAM-**2** (right). Note the differences in the relative intensities of various peaks, particularly those labeled, in the calculated and experimental patterns of the anhydrous form.

All the SAMs used herein have the same two-dimensional lattice ($a_1 = a_2 = 4.97$ Å and $\alpha = 120°$).[21] The program determines the lattice registry by rotating an overlayer lattice (b_1, b_2, β) on a substrate lattice (a_1, a_2, α) through a series of azimuthal angles (θ; see the Supporting Information, Figure S7). For each azimuthal angle, the program calculates a dimensionless potential (V/V_o), whose value depends on the type of epitaxy between the two lattices (Table S2). Analysis of the results shows that the {200}, {201}, {111}, and {011} faces of the anhydrous form, and only the {011} and {10$\bar{1}$} faces of the monohydrate form exhibit ideal coincident epitaxy; other faces deviate from the ideal value of $V/V_o = 0.50$ (Table S3 in the Supporting Information). Of all the faces that show the ideal V/V_o value, the {200} faces of the anhydrous form have the smallest supercell area; that is, these faces exhibit the best epitaxial match with the SAM substrates.

Unlike in the anhydrous form, all the donors and acceptors in the monohydrate form of theophylline are involved in hydrogen bonding. The water molecule provides the missing hydrogen-bond donors; that is, there is no shortage of donors within the crystal structure of the monohydrate. In terms of geometric epitaxy, the {101} faces displayed the smallest supercell area (Table S3). Figure 5 shows that the molecules are arranged in a corrugated pattern at the (101) plane, such that =CH and CH_3 groups are exposed. Hydrogen-bond acceptors are not easily accessible at this plane; that is, the {101} faces are less likely to be nucleated by hydrogen-bonding SAM-**3**, SAM-**4**, and SAM-**5**. Further analysis showed that all the growth faces in the monohydrate display structural features (corrugated packing or inaccessible donors, Figure S9) that are not conducive to template-induced nucleation by the hydrophilic SAMs. The exclusive growth of the monohydrate form on hydrophobic SAM-**1** and SAM-**2** is difficult to rationalize. As noted above, the (101) and other planes expose =CH and CH_3 groups, which can interact favorably with CH_3-terminated SAMs. In addition, the growth of the anhydrous form is probably hampered in the presence of hydrophobic SAMs, leading to the enhanced growth of the monohydrate form. Powder X-ray diffraction

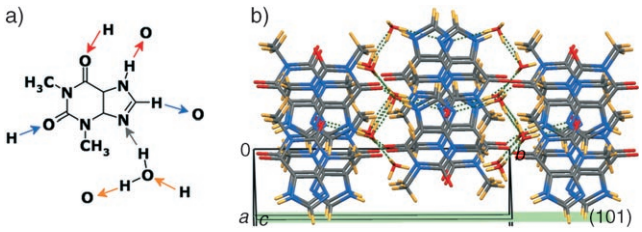

Figure 5. The crystal structure of the monohydrate form of theophylline.[16] a) The hydrogen bonds around a single molecule; the red, yellow, and blue arrows indicate three distinct hydrogen bonds. Note that all potential donors and acceptors participate in hydrogen bonding. b) The corrugated packing of the molecules; C gray, H yellow, N blue, O red; the green dotted lines indicate hydrogen bonds. Note the absence of exposed acceptors at the (101) interface. One of the hydrogen atoms of the water molecule is disordered.

analysis showed that the relative intensities of the experimental and calculated (with no preferred orientation of crystals) patterns are similar in the case of the monohydrate (Figure 4). This observation indicates that face-selective crystal growth does not occur in the case of the monohydrate. In other words, all faces of the crystals of the monohydrate form exhibit similar growth rates on hydrophobic surfaces.

We showed that hydrogen-bonding SAMs act as templates for the selective growth of the thermodynamically less stable anhydrous form of theophylline. We believe that several properties inherent to the anhydrous form led to this selectivity: an imbalance in the numbers of hydrogen-bond donors and acceptors, a layered arrangement of molecules that exposes the excess acceptors at the largest growing faces, and a serendipitous coincident epitaxy with the hydrophilic SAMs. Polymorph selection by surface templates is currently in the discovery phase. We hope that our work and the recent work by others will enable future research in this area to enter the design phase, where templates can be created for the growth of a desired polymorph. We discovered a previously unidentified or unnoted problem in the surface-induced crystallization of polymorphs: the growth of unwanted polymorphs on adventitious templates. Crystallizations are typically performed in a container, and the surface of the container is always a potential nucleation site for polymorphs. We are currently exploring several approaches to minimize or eliminate this problem of polymorph growth on surfaces other than the desired templates.

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[19] Glass slides with exposed silanol (SiOH) groups were prepared by treating freshly cut slides with an air plasma (in an SPI-III plasma etcher) for 45 s. These slides are similar to SAM-**3**, SAM-**4**, and SAM-**5** in terms of their hydrophilicity and hydrogen bonding. They, however, lack the long-range two-dimensional order and periodicity that are characteristic of the SAMs.

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[21] Thiol SAMs are known to form domains of 100–200 μm^2 that have long-range order.[17] The epitaxy calculations assume that templated nucleation occurs on these domains.

