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Polymorphism

Selective Growth of a Stable Drug Polymorph by Suppressing the Nucleation of Corresponding Metastable Polymorphs

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Early discovery of thermodynamically stable drug polymorphs is critical in pharmaceutical development to avoid formulation problems and potential withdrawal of the life-saving drugs from the market.[1] Tales of disappearing polymorphs are well known in chemical literature;[2] most of these tales are attributable to the late-stage appearance of a thermodynamically stable polymorph that replaced the previously existing metastable form.[3] Ritonavir, a protease-inhibitor drug that had to be recalled after being on the market for two years, exemplifies this problem.[1] In this study, we report the unique ability of perfluoroalkyl-terminated silane monolayers[4] in promoting the exclusive growth of the stable polymorph (γ form) of indomethacin, a nonsteroidal anti-inflammatory drug.[5] This selective growth is promoted not by the enhanced nucleation of the γ form, but by the suppressed nucleation of the metastable polymorph (α form). We also show that silane monolayers fabricated on the surfaces of glass vials (as opposed to monolayers fabricated on glass slides) minimize concomitant crystallization of polymorphs.

Several researchers have used structured (thiol self-assembled monolayers,[6] Langmuir monolayers,[7] and single-crystal faces[8]) and nonstructured (silane monolayers[9] and polymer particles[10]) surfaces to study the crystal growth of organic and inorganic compounds.[11] Polymorph selectivity is observed on structured and nonstructured surfaces; chemical complementarity assisted by some level of geometric complementarity at the growth interface is assumed to be responsible for the observed selectivity.[12] Indomethacin possesses several functionalities (carboxy, tertiary amido, methoxy, chloro; Figure 1a) and we have explored the influence of silane monolayers bearing different functional groups (**3**–**9**, Figure 2) on its crystal growth. In all the experiments, we used bare-glass (**1**) and plasma-oxidized-glass (**2**) substrates as controls.

Indomethacin crystallizes concomitantly as α and γ polymorphs from ethanol.[13] These two polymorphs have distinct crystal structures (α: $P2_1$, $Z'=3$, γ: $P\bar{1}$, $Z'=1$),[14] melting points (α: 153 °C, γ: 158 °C), and morphologies (α: needles, γ: plates). They can be identified by powder X-ray diffraction (PXRD), IR spectroscopy, differential scanning calorimetry (DSC), and optical microscopy.[15] In the beginning, we studied the effect of silane substrates **1**–**9** on the crystal growth of indomethacin by using the growth method 1 (Figure 1b). In this method, we prepared silane monolayers on glass slides, placed these slides in glass vials containing ethanolic solutions of indomethacin, and grew crystals by slow evaporation of the solvent. Repeated experiments by using method 1 and substrates **1**–**9** led to three consistent results: 1) both α and γ polymorphs crystallize concomitantly on substrates **1**–**8** (Figure 3a), 2) on substrate **9**, crystals of γ polymorph are formed predominantly (Figure 3c), and 3) crystals of α polymorphs are always formed on the vial surfaces (Figure 3b and d), especially on the walls of the vials.

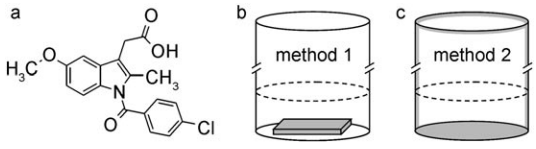
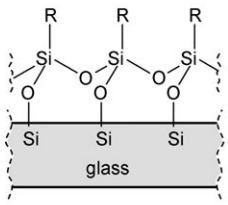


Figure 1. a) Molecular structure of indomethacin. b,c) Two methods of crystal growth used in this work. Shading indicates silane monolayers, dashed lines show the air–solution interface.



3: R = $-CH_2CH_2CO_2CH_3$
4: R = $-CH_2CH_2CH_2C{\equiv}N$
5: R = $-CH_2CH_2CH_2Cl$
6: R = $-C_6H_4CH_2Cl$
7: R = $-(CH_2)_9CH{=}CH_2$
8: R = $-(CH_2)_{17}CH_3$
9: R = $-(CH_2)_2(CF)_4CF_3$

Figure 2. Schematic illustration of silane substrates used in this work.

Figure 4 shows the relative amount of the γ polymorph obtained on substrates **1**–**9** in eight different experiments. Initially, we characterized α and γ polymorphs by using PXRD, IR spectroscopy, and DSC (see Figures S2–S4 in the Supporting Information). With experience, however, we could readily distinguish between the two forms by optical microscopy. To obtain the plots in Figure 4, we separated the crystals of the γ polymorph from the slides and vials and weighed the quantities of the two polymorphs on an analytical balance. Though this method is approximate, it is rapid and avoids the cogrinding of samples, which may result in the unintended phase transition between the two polymorphs.[16]

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Supporting information for this article, including details for the preparation of silane monolayers on glass slides and vials, crystallizations in different conditions, characterization of indomethacin polymorphs by IR spectroscopy, DSC and PXRD, and enlarged images of Figures 3 and 5, is available on the WWW under http://www.angewandte.org or from the author.

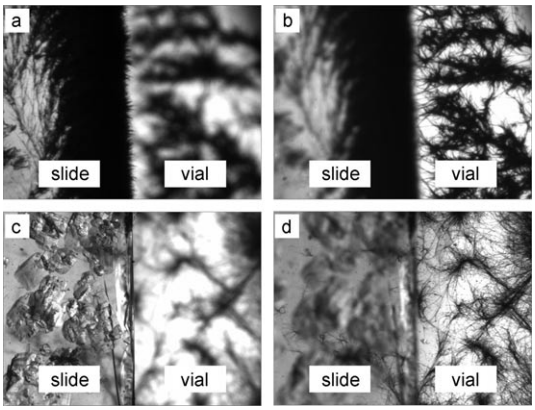

Figure 3. Indomethacin crystal growth by using method 1. Slides functionalized with silane monolayers are on the left side of the images. The right portions of the images show the bottoms of the vials not covered by the slides. a,b) Crystal growth on **5** monolayers with focus on the slide (in a) and on the vial (in b). Notice the predominant growth of α polymorph on both locations. c,d) Crystal growth on **9** monolayers with focus on the slide (c) and on the vial (d). Notice the predominant growth of γ polymorph on the slide and α polymorph at the bottom of the vial. See also Figure S7 in the Supporting Information.

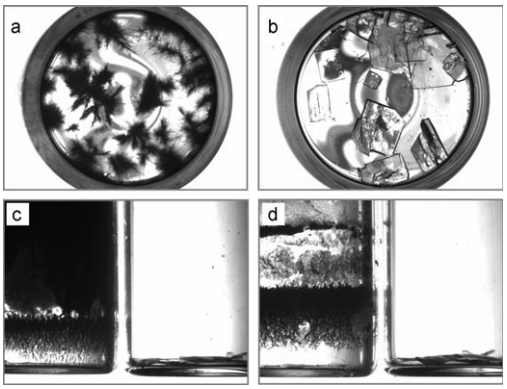

Figure 5. Indomethacin crystal growth by using method 2. a,b) View perpendicular to the bottom of the vial showing the crystal growth on **5** (a) and **9** (b) monolayers. In this zoomed out view, only α crystals are seen in (a). At a closer view, this vial contains approximately 2% of γ crystals. Note the exclusive growth (and larger size) of γ crystals in (b). c,d) View parallel to the bottom of the vial showing the crystals grown on **5** (left vial) and **9** (right vial) monolayers in ethanol (c) and acetonitrile (d) solutions. Note the rampant crystal growth of the α form on the walls of **5** vials and the absence of crystal growth walls in **9** vials. Notice the γ crystals at the bottoms of **9** vials.

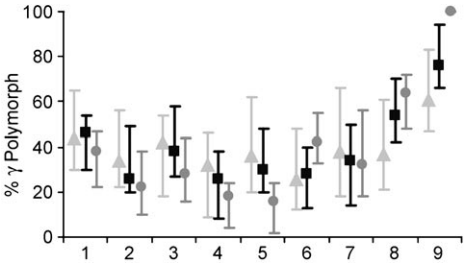

Figure 4. Relative amount of γ polymorph grown on substrates **1**–**9** by using method 1 (▲: slides plus vials; ■: slides only) and method 2 (●). The position of the marker indicates the average value from eight experiments; error bars show the highest and lowest amounts of the γ polymorph obtained. Note the exclusive growth (no error bars) of γ polymorph on **9** monolayers by using method 2.

Such cogrinding is required for quantification by DSC, PXRD, and IR spectroscopy.

The analysis of the total solid material in each vial (Figure 4, ▲) shows that the amount of γ polymorph in vials containing slides of **9** is greater when compared with other vials. This result becomes more prominent when the analysis is restricted only to the material that is present on the slides (Figure 4, ■). These findings suggest that **9** surfaces may be responsible for the enhanced growth of the γ polymorph. We reasoned that nucleation of the α polymorph on vial surfaces (and probably also in bulk solution) is responsible for the concomitant crystallization of the two polymorphs in vials containing **9** slides.

To eliminate the competing influence of two different surfaces on the crystal growth, we fabricated the silane monolayers directly on the inner surfaces of the glass vials and used these vials for crystal growth (Figure 5).[17] This is our growth method 2 (Figure 1c); the circles in Figure 4 show the results from this method. Although a mixture of two polymorphs crystallizes in control vials **1**–**2** and in vials functionalized with monolayers **3**–**8** (with some differences in the relative amounts of two forms), vials coated with **9** monolayers yielded the γ polymorph exclusively in eight different experiments. Similar results were obtained when crystallizations were carried out in acetonitrile[18] or other altered conditions.[19]

Why does the γ form grow preferentially and exclusively in vials containing perfluorinated monolayers? The following experimental observations help understand the selectivity: 1) Except in vials functionalized with **9** monolayers, crystals of mostly α-polymorph grew on the vial walls as the solvent front evaporated (Figure 5c–d). 2) Crystals (of γ polymorph) appeared after a significantly longer time interval in **9** vials. When crystallizations were carried out with 25 mM ethanolic solutions of indomethacin at 20°C, crystals of the α polymorph appeared on the walls of vials **1**–**8** in 14–20 h, whereas the crystals of γ polymorph appeared at the bottom of the **9** vials in 30–56 h (Figure 5b). 3) The total number of crystals in a given **9** vial is much smaller than in other vials. In addition, individual crystals are larger and well formed in **9** vials.

These observations can be explained if we surmise that the crystal growth of α and γ polymorphs follows the Ostwald's rule of stages.[20] Accordingly, the nuclei of the α form (kinetic polymorph) are formed in preference to the γ form (thermodynamic polymorph) at the onset of saturation. Crystals of α polymorph grow on nonperfluorinated substrates **1**–**8** because these substrates allow the adsorption of α nuclei on their surfaces and promote the heterogeneous nucleation of the α form. In contrast, the perfluoroalkyl monolayers **9** inhibit the attachment of kinetic nuclei to the surface, thereby suppressing their heterogeneous nucleation and further crystal growth of the α polymorph. With time, however, the nuclei of thermodynamic γ polymorphs are formed in solution; as the solvent evaporates, crystals of γ polymorph grow

while the nuclei of α polymorph dissolve in solution. This phenomenon, the growth of a stable form at the expense of a metastable form, is known as Ostwald ripening.[20] The nonstick nature of perfluoroalkyl surfaces (e.g., Teflon) is well established. In this work, we used nonstick surfaces to thwart the nucleation of the metastable polymorph; in doing so, these surfaces promote the growth of the stable polymorph.

We believe that the new method described herein—crystal growth in vials exposing nonstick surfaces—will find widespread applications in pharmaceutical crystallization and polymorphism. Unlike the surface-enabled crystal-growth methodologies developed before, the current method does not require the knowledge of specific interfacial interactions. Thus, this new method can be applied to any solid drug even if its structural, morphological and other physical properties are unknown. We expect that the use of this method will significantly increase the probability of finding the thermodynamically stable drug polymorphs at the early stages of pharmaceutical development.[21] This method is also directly applicable and relevant to the high-throughput screening of crystallizations, which is widely used in the current pharmaceutical industry. Furthermore, the silane monolayers are robust (when compared with thiol monolayers on metal surfaces) and they are less likely to contaminate crystalline materials grown by using the current method. We are currently applying this new method to several polymorphic drugs to test its generality and wider applicability. Preliminary results suggest that this method is also applicable to inorganic salts and organic compounds with heteroatom-containing functional groups.

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[17] This method also removes the high energy interfaces occurring at the slide edges. Crystallization is often initiated at the edges; eliminating the edge effects is therefore important in experiments involving surface-induced nucleation.

[18] It was reported in Ref. [13] that only the α form crystallizes from acetonitrile solutions. In our experiments, however, both α and γ polymorphs crystallized concomitantly from this solvent placed in control vials **1**–**2** or vials functionalized with **3**–**8**

monolayers. In contrast, crystallization in acetonitrile from **9** vials yielded the γ polymorph exclusively (Figure 5d).

[19] The crystal growth experiments described so far used ethanol solutions made from the γ polymorph of indomethacin. We also carried out crystallizations from ethanol solutions that were made from α-polymorph. In separate experiments, we performed crystallizations by evaporating ethanol at a much slower rate and also at 0 °C. In all these cases, we obtained similar results: a) crystals of α polymorph always grew on the walls of vials **1**–**8**; b) no crystal growth occurred on the walls of **9** vials; and c) crystals of only γ polymorph were grown in **9** vials. See the Supporting Information for details.

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[21] Industrial crystallizations use large containers and in this case the current method cannot be applied directly. We found that crystallizations carried out in **9** vials with smaller surface-to-volume ratios led to the concomitant crystal growth of α (4–20%) and γ polymorphs (80–96%). For details, see Figure S5 and the related discussion in the Supporting Information. The primary application of this method lies in the discovery of stable polymorphs by using smaller vials (for example in a high-throughput setting). If necessary, the crystals obtained in small vials can be used as seeds for further growth in larger containers.

