

## One Polymorph and Various Morphologies of Phenytoin at a Silica Surface Due to Preparation Kinetics

Heike M. A. Ehmann,<sup>†</sup> Ramona Baumgartner,<sup>†</sup> Daniela Reischl,<sup>†</sup> Eva Roblegg,<sup>†,‡</sup> Andreas Zimmer,<sup>†</sup> Roland Resel,<sup>§</sup> and Oliver Werzer\*,<sup>†</sup>

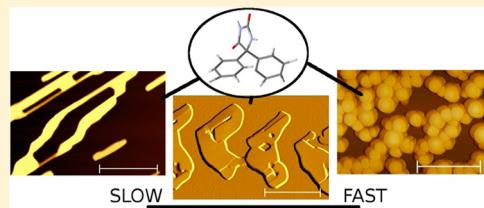
<sup>†</sup>Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology, Graz University, 8010 Graz, Austria

<sup>‡</sup>Research Center Pharmaceutical Engineering GmbH, 8010 Graz, Austria

<sup>§</sup>Institute for Solid State Physics, Graz University of Technology, 8010 Graz, Austria

### Supporting Information

**ABSTRACT:** The preparation of solid crystalline films at surfaces is of great interest in a variety of fields. Within this work the preparation of pharmaceutically relevant thin films containing the active pharmaceutical ingredient phenytoin is demonstrated. The preparation techniques applied include drop casting, spin coating, and vacuum deposition. For the solution processed samples a decisive impact of the solution concentration and the applied film fabrication technique is observed; particular films form for all samples but with their extensions along different crystallographic directions strongly altered. Vacuum deposition of phenytoin reveals amorphous films, which over time crystallize into needle-like or particular-type structures whereby a nominal thickness of 50 nm is required to achieve a fully closed layer. Independent of all preparation techniques, the resulting polymorph is the same for each sample as confirmed by specular X-ray diffraction scans. Thus, morphologies observed via optical and atomic force microscope techniques are therefore a result of the preparation technique. This shows that the different time scales for which crystallization is obtained is the driving force for the various morphologies in phenytoin thin films rather than the presence of another polymorph forming.



### INTRODUCTION

Large quantities of newly developed pharmacologically active molecules suffer from poor water solubility resulting in insufficient biopharmaceutical properties since drug solubility and dissolution rates are frequently the limiting steps in intestinal drug absorption.<sup>1</sup> For reasons of improving drug efficacy and safety, there is an enormous need in innovative formulation platforms and drug delivery technologies to overcome these limitations. Presently, numerous strategies focus on that issue, including the formation of water-soluble inclusion complexes,<sup>2</sup> self-(micro)emulsifying drug delivery systems,<sup>3,4</sup> solid dispersions,<sup>5,6</sup> and solid solutions<sup>5</sup> or nano-suspensions.<sup>7–9</sup> Among the mentioned approaches for enhancing drug solubility and thus systemic absorption, the modification of particle size,<sup>10</sup> crystalline structure or polymorph,<sup>11,12</sup> and morphology are quiet effective procedures and overcome some of the drawbacks of other formulation technologies.<sup>13</sup>

Recently, the formulation of poorly soluble pharmacologically active compounds as nanosized crystals gained popularity<sup>10</sup> with several products reaching the market.<sup>14</sup> The beneficial property of nanocrystals is the significant increase in their specific surface area per mass compared to conventional large sized crystals. This causes a considerably higher dissolution rate<sup>15</sup> and saturation solubility.<sup>10,16</sup> Furthermore, nanocrystals possess a high phase stability.<sup>7</sup>

The application of drug molecules via thin films, typically present in buccal or transdermal patches, has a great advantage as the acidity of the gastrointestinal tract and the first pass effect in the liver can be bypassed.<sup>17,18</sup> This is of great importance for many newly developed or already well-established drug molecules. Anyway, the preparation of thin active pharmaceutical ingredient (API) films is typically distinct from standard preparation routes in the bulk. In addition, the shape and polymorph forming on the production may be altered as surface-API interactions induce the molecules to assemble in a structure which is different from the bulk.<sup>19–22</sup> Even preferred alignments of the crystallites with respect to the surface can be found.<sup>23,24</sup> While amorphous solid states are in general favorable in terms of solubility, they often lack long-term stability<sup>25</sup> suffering from recrystallization processes during storage which could lead to a loss of the beneficial solubility behavior. In addition, the chemical stability may be reduced, and therefore crystals are often preferable in solid pharmaceutical formulations.

In this work the preparation of various thin films containing a model drug molecule, i.e., phenytoin, is demonstrated. Within the entire study only one crystalline polymorphic structure is obtained, while the different preparation routes have a decisive

**Received:** September 17, 2014

**Revised:** November 3, 2014

**Published:** November 25, 2014

influence on the forming morphology. These observed morphologies are investigated via optical and/or atomic force microscopy. The crystalline structures are further investigated by X-ray diffraction experiments. The resulting structures are discussed in terms of the kinetics of the preparation processes that is sufficient to understand their formation and can be expected to be similar for a variety of similar molecules.

## MATERIALS AND METHODS

5,5-Diphenyl-2,4-imidazolidinedione (phenytoin) has a chemical formula of  $C_{15}H_{12}N_2O_2$  and its structure is depicted in Figure 1.

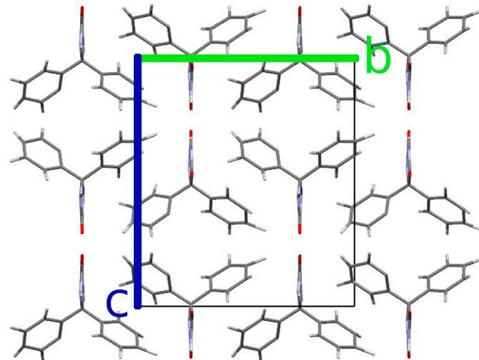


Figure 1. Phenytoin molecules in the unit cell viewed along the *a*-axis.

The powder was purchased from Sigma (Germany, pharm. grade) and used without further treatment. Various solvents were purchased from various suppliers in spectroscopic grade. Solution of the API and the solvent were prepared in various concentrations at 30 °C and stirred 8 h prior to the experiments.

As substrates, conventional glass slides (Roth, Germany) were cut in  $2.5 \times 2.5 \text{ cm}^2$  pieces. Prior to the experiments, the substrates were cleaned in a 1:1 (v/v) ethanol/acetone solution in an ultrasonic bath and were subsequently rinsed with isopropanol. Finally, the pieces were dried under a nitrogen stream. Such surfaces are slightly hydrophilic with a water contact angle of about 35°.

Drop casted films were prepared by placing a defined amount of 200  $\mu\text{L}$  solution on the clean glass slides. Care was taken about the leveling of the surface to minimize inhomogeneous drying due to gravity causing the solution to run into one corner of the sample. A cover was used to control the solvent evaporation rate. Spin coated samples were prepared by a standard spin-coater. The samples were mounted using a double-sided sticking tape to minimize solvent removal by the vacuum systems. A rotation speed of 15 rps for 30 s was sufficient to achieve homogeneous films as shown previously.<sup>10</sup> The vacuum deposition of phenytoin onto the precleaned silica surfaces was performed with a custom-made setup. A vacuum of  $10^{-4}$  mbar and a sublimation temperature of 110 °C result in a deposition rate of 1 nm/min. The substrates were kept at room temperature during the deposition. The obtained films at the silica surface were completely amorphous after the deposition. As will be mentioned later, these films crystallize at ambient condition within 24 h.

Specular X-ray diffraction (XRD) scans were performed with a Siemens D500 diffractometer. The machine was setup in a Bragg–Brentano configuration<sup>26</sup> with primary, secondary slits and a soller slit. The radiation was provided by a copper sealed tube with a wavelength ( $\lambda$ ) of 0.154 nm and monochromatized with a graphite single crystal. The angular specular scans ( $\theta$ -scans), which provide only information on the net planes which are parallel to the surface, are transferred into scattering vector ( $q_z$ ) notation by using  $q_z = 4\pi \sin(\theta) / \lambda$ .

Atomic force microscopy (AFM) height images were taken with a Nanosurf Easyscan 2 (Switzerland) machine in noncontact mode. The cantilevers were Tap190 (Budgetsensors, Bulgaria) with a nominal frequency of 190 kHz. The data were corrected for artifacts and

plotted with the software package Gwyddion.<sup>27</sup> Optical microscope investigations of the films were performed with a Zeiss Axio Vision microscope between crossed polarizers.

## RESULTS

**Drop Cast: Ethanol.** In Figure 2 optical micrographs of phenytoin drop cast films from a 2.0 wt % ethanol solution (6.4

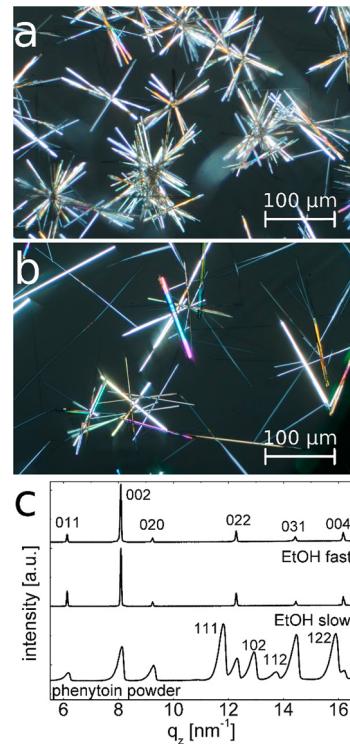


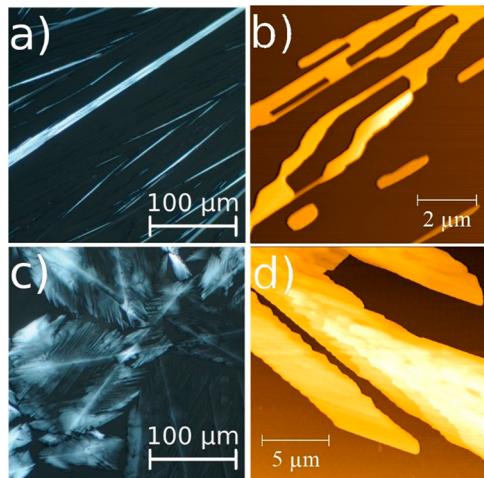
Figure 2. Optical micrograph images of drop cast phenytoin from a 2.0 wt % ethanol solution after fast (a) and slow (b) solvent evaporation. Corresponding X-ray diffraction pattern of these two samples and an experimental powder pattern of the as-purchased phenytoin powder (c). Data are shifted for clarity.

mM) are shown for fast (a) and slow evaporating (b) solvent. Within the sample prepared via fast solvent evaporation (<1 min) various unidirectional grown needle-like structures are present with maximal extension of about 100  $\mu\text{m}$ . The rod-like crystalline structures show connection points at common centers. As the evaporation of the solvent was slowed down by a cover, meaning that the solvent evaporation takes 30 min rather than 1 min, the morphology remains similar (Figure 2b). However, the elongation of the individual rods is increased; a maximal extension of 300  $\mu\text{m}$  is observed which is about three times larger compared to the sample prepared on fast evaporation. In addition, the needles do not show common centers, and the number of intersecting points is reduced.

The corresponding specular X-ray diffraction patterns are shown in Figure 2c. The scans reveal various peaks over the entire scan range. A comparison of this experimental data with a powder pattern taken for the as-delivered bulk material shows that the drop cast films have the same polymorphic structure; i.e., the peak positions of the observed peaks are identical. To our knowledge, only one polymorphic modification of phenytoin exists in the literature;<sup>28</sup> whereby the phenytoin molecules pack in an orthorhombic unit cell with  $a = 0.62 \text{ nm}$ ,  $b = 1.36 \text{ nm}$ , and  $c = 1.55 \text{ nm}$ , which is in excellent agreement

with the specular scans and the powder pattern in this study. The pattern of the drop casted films and the powder pattern reveal that Bragg reflections connected to Miller indices ( $hkl$ 's), whereby the reflections with  $h$  equal to 1 are absent in the drop casted films. This is due to the rod-like structure assembling nearly parallel to the surface; Miller indices along the long needle axis, which lies normal to the surface, are not accessible within this kind of measurement. Typically within a specular scan, only netplanes that are parallel to the surface are able to contribute to a pattern with a Bragg peak. While most of the reflections intensities are in good agreement with respect to the powder pattern, the 031 peak is relatively weak. This on the one hand may be a result of a slight texture being present, meaning that a preferred orientation exists disfavoring the 031 being parallel to the surface. On the other hand, this may be a result of the 031 netplane being less likely to develop during the crystallization process.

Reducing the solute concentration in ethanol to 1.0 wt % (3.2 mM) results in a deviation of the crystal morphology. At a slow evaporation rate ( $\sim 30$  min), elongated structures are observed within the optical microscopy image which cover the entire surface (see Figure 3a); individual rods show extensions

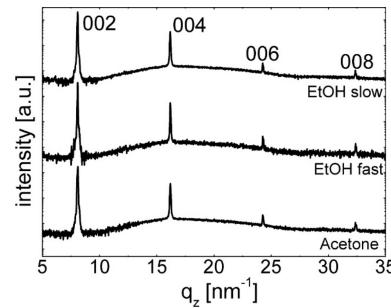


**Figure 3.** (a–d) Optical microscopy images (left row) and AFM height images of phenytoin crystals obtained on slow (top) and fast (bottom) solvent evaporation from a 1 wt % ethanol solution.

of more than  $200 \mu\text{m}$ . Furthermore, the individual rods are a result of the bigger rods forming side-branches. An AFM height image of this sample, taken at a spot at which a small rod is present, reveals that the elongated structures have vacancies. Most likely the phenytoin molecules adapt positions at the front of the crystal rather than the perpendicular direction; a meanwhile perpendicular or inclined growth is followed by a growth along the long rod axis leaving vacancies.

Reducing the evaporation time of the 1.0 wt % ethanol solution to about 1 min results in the formation of three-dimensional (3D) dendritic structures (see Figure 3c,d). From a common center, long rod-like structures evolve which enclose an angle of  $45^\circ$ . These rod-like structures are flanked by side branches, which grow again  $45^\circ$  inclined with respect to the long rod-like structures. A corresponding AFM height image reveals that individual branches consist of parallel sheets that stack on top of each other. This results in a 3D growth which is different to the samples prepared at slow evaporation speeds. In addition, vacancies within a single branch are absent.

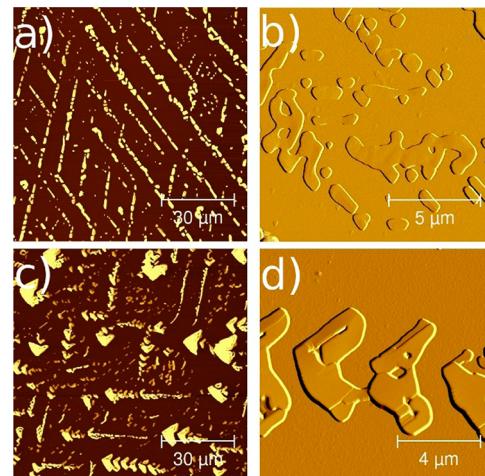
Specular X-ray diffraction scans of the samples prepared from the 1.0% wt solution reveal just peaks corresponding to the 002 and higher order reflections independent of the solvent removal time (see Figure 4). This means that the phenytoin crystals



**Figure 4.** Specular X-ray diffraction scans of phenytoin prepared from 1.0 wt % ethanol and acetone solutions, respectively. Data are shifted for clarity.

preferentially organize with respect to the surface; i.e., the (001) netplane is in contact with the silica surface which in general is referred as texture. This texture is further verified by grazing incidence diffraction measurements (see Supporting Information) showing that the mosaicity within the sample is also low.

**Drop Cast: Acetone.** Changing the solvent to acetone results in distinct structures at the silica surface. At a concentration of 1.0 wt % (3.2 mM) and an evaporation time of about 5 min a two-dimensional (2D) network of phenytoin forms with an extension of over more than  $100 \mu\text{m}$  (see AFM height image in Figure 5a). (Lower evaporation rates are not



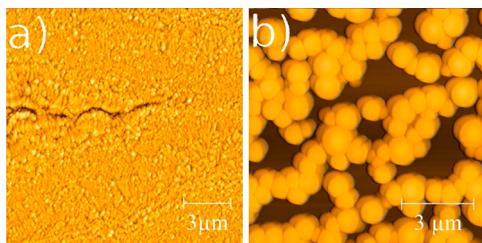
**Figure 5.** AFM height (left) and amplitude (right) images of phenytoin crystals obtained from a 1.0 wt % (a, b) and 1.5 wt % (c, d) acetone solution.

accessible within this setup as the vapor pressure is higher than those of EtOH.) Preferred growth directions can be identified with each “side direction” being  $45^\circ$  inclined to another. A higher resolution image of the sample reveals vacancies along the growth direction. In addition, the shape of the crystallites is more flake-like rather than rod-like compared to the ethanol casted sample. Surprisingly, even though a long-range order seems to exist the individual crystals are not interconnected (see Figure 5).

Increasing the API concentration in acetone to 1.5 wt % (4.8 mM) results again in the formation of ordered structures at an evaporation time of 5 min. The shape of the crystals, however, has drastically changed (compare Figure 5, panels a and c). Banana- or boomerang-like shaped crystals arrange in common directions. Two main directions (one parallel to the image and one that is inclined by 85°) are observed. Interestingly, while the boomerang shapes are reproduced, the size of the individual boomerangs decreases steadily for adjacent boomerangs. Up to nine boomerangs can be observed in close vicinity behaving in this manner. Similarly to the sample prepared from lower concentrations, the crystallites show vacancies. Interconnections are present for some of the neighboring crystallites, but many boomerangs are not connected (see Figure 5d) raising the question of the factors responsible for this ordered growth.

The X-ray diffraction experiment of the sample prepared from 1.5 wt % phenytoin–acetone solution shows that the polymorphic structure is identical to the ethanol sample prepared from low concentration of 1.0 wt %. In addition the same preferred 001 texture is present (see Figure 4 and Supporting Information for grazing incidence data).

**Spin Coating: Acetone and Ethanol.** Drop casting solutions result in relatively slow evaporation of the solvents, and at least 1 min is required for the processing. By using a spin casting process the solvent evaporation is strongly enhanced. Examples of phenytoin spin coated from the 1.5 wt % acetone solution and the 1.0 wt % EtOH solution are shown in Figure 6.



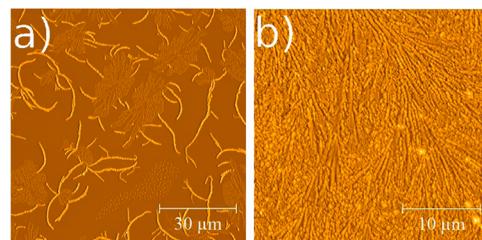
**Figure 6.** AFM height images of phenytoin spin coated from 1.5 wt % acetone solution (a) and 1.0 wt % ethanol solution (b).

The AFM height image of the acetone sample exhibits particles packing together to form a homogeneous film. A power spectral density investigation shows that the particles have a size of about 400 nm (see Supporting Information or elsewhere).<sup>10</sup>

Thin film preparation of phenytoin from a 1.0 wt % EtOH solution via spin coating reveals the formation of slightly interconnected particles. However, other than the sample prepared from the acetone solution, this sample reveals surface areas without phenytoin and the substrate is visible. The size of the particles compared to the acetone sample is also strongly increased and about 750 nm (see Supporting Information).

X-ray investigation of the spin coated sample reveal that a preferred alignment of the crystallites is absent. Grazing incidence X-ray diffraction measurements show a random alignment of the crystallites takes place typically for a powder-like behavior (see Supporting Information) but with the polymorph remaining the same.

**Physical Vacuum Deposition.** The samples shown above were prepared from solution based techniques either via drop casting or spin coating. Anyway, in Figure 7 crystalline thin films of phenytoin prepared by physical vapor deposition are shown. At a rate of 1 nm/min phenytoin assembles as an amorphous structure at the silica surface. The initial film is



**Figure 7.** AFM height images of phenytoin crystal morphologies developed 24 h after vacuum deposition for a 15 nm (a) and a 50 nm (b) nominal thick film.

homogeneous, flat, and amorphous (shown in the Supporting Information and elsewhere<sup>25</sup>). After 24 h at ambient conditions these featureless amorphous films have developed into crystalline structures (see Figure 7); i.e., spontaneous crystallization took place. At a low nominal thickness, i.e., short deposition time, an amorphous (nominal) layer thickness of 15 nm is initially achieved. As the sample is left for 24 h at ambient conditions, spontaneous crystallization is induced whereby three different types of structures can be identified in this thin film (Figure 7a). The most prominent structure hereby consists of long kinked needles with extensions of various micrometers, a height of 150 nm, and a width of about 1 μm. Further, flat spherulite-type structures can be identified, whereby the flat branches have a common center with a height of about 50 nm. In addition, drop-like formations can be seen which are typical for amorphous phenytoin as observable in the left middle of Figure 7a. This means that the crystallization was not fully developed throughout the film in this thin film after the investigated period. The X-ray investigations did not show any crystalline or amorphous diffraction signal from phenytoin, indicating that the film was too thin to provide sufficient diffraction power.

Increasing the deposited layer thickness to 50 nm results again in an initial amorphous film, but after 24 h crystals have formed with the morphology being different from the thin film of initial 15 nm thickness (see Figure 7b). Densely packed rods, typically spherulitic in nature, run along the surface. Furthermore, the entire film is covered with these structures meaning that the amount of phenytoin was sufficient to achieve full coverage even after crystallization. Specular X-ray diffraction measurements of the samples show that for the thicker film a preferred 001 orientation is again present (see Supporting Information).

## ■ DISCUSSION

The various techniques used for the thin film preparation result in the formation of various morphologies at the silica surface. While all techniques show that phenytoin crystallizes in an orthorhombic unit cell with  $a = 0.62$  nm,  $b = 1.36$  nm, and  $c = 1.55$  nm with a defined packing (see Figure 1), the time frame for crystallization differs significantly and thus the morphology is altered. Using a vacuum deposition technique results in the phenytoin films being amorphous (see Supporting Information). The sublimation of phenytoin and its fast adsorption on the silica surface mean that the molecules do not have sufficient time to assemble in a crystalline structure. A solid amorphous state means that molecular movements are slowed down, i.e., the translation and/or rotation, which are required to adapt at a crystalline site, are hindered by adjacent molecules or the solid silica surface, which helps the film to remain amorphous for a

long period. Anyway, this amorphous state is metastable and transfers into a crystalline state; after 24 h a crystalline film is obtained. Within a pharmaceutical application such amorphous states would be preferable due to their enhanced dissolution properties, but as the stability is only given for a couple of hours, these films are not suitable, as long shelf life times (up to two years) are required. As seen from the results, amorphous films were only accessible for vacuum-deposited phenytoin. All other solution-processed films showed their crystalline structure directly after the preparation process. It might be possible to obtain amorphous films using a fast evaporating solvent, like chloroform, within a spin-casting process, but this was not tested as such a solvent should be prevented in pharmaceutical applications.

Within recent experiments it was shown that this amorphous state can be altered faster either via mechanical induction with an AFM tip<sup>25</sup> or via solvent annealing.<sup>29</sup> Within the first experiments, the AFM tip induces a perturbation that is sufficient for nucleation and a subsequent crystal growth. The resulting AFM induced morphologies strongly depended on the layer thickness of the amorphous film. Using a vapor annealing process, morphologies different from the once observed here (or induced with the AFM) are obtained. In a vapor annealing process, the sample is in contact with a solvent vapor that interacts with the phenytoin molecules. As such, a change of the solvent results in a change in the crystal morphology with the solvent quality having a decisive impact;<sup>30</sup> for instance, a water vapor result in structures that are similar to the spin coated samples shown in this work, while alcohols result in large single crystals well separated on the silica surface. From this it follows that the amorphous state is highly unstable and can be easily transferred into a crystalline state of various morphologies.

Spin coating is a fast processing procedure leaving the system hardly any time for crystalline assembling. However, as a solvent is present during the processing, the diffusion rate of the API molecules in the film is enhanced compared to the vacuum deposited thin films. This allows the molecules to assemble in the same crystalline state independent of the solvent in use. The resulting morphologies are distinct for the two different solvents. Films with small particles develop during the preparation from acetone solutions, while in EtOH bigger and more separated disc-like shapes are present. The differences in their morphologies are most likely a result from the different wettability of the two solvents; i.e., acetone solutions fully wet the surface, while EtOH has a nonvanishing contact angle due to the interplay of cohesion and adhesion. This results in the drying of the film being altered. In acetone a single drop homogeneously spreads and dries over the entire substrate surface, while the formation of many small droplets in EtOH is a result from dewetting, in which individual crystals form. In addition, the vapor pressures of the two solvents are markedly different ( $p_{(\text{acetone})} = 230 \text{ Torr}$ ,  $p_{(\text{EtOH})} = 59 \text{ Torr}$ )<sup>31</sup> meaning that evaporation of acetone is faster compared to EtOH, providing the API molecules less time for the assembling in acetone compared to EtOH. As a result, more and smaller crystallites form when processed in acetone, while when EtOH is used, it is likely that the drop-type structures consist of single crystals that form due to the longer evaporation time. Recent experiments also revealed that the usage of the spin coating technique for the sample preparation increases the maximum solubility of phenytoin in an aqueous environment and the dissolution rate is strongly enhanced compared to a bulk powder.<sup>10</sup> Both effects were explained in

terms of the particle size being much smaller and thus the surface area being increased in accordance with theory.<sup>32</sup>

The drop casting process is the most versatile preparation technique whereby the morphology of phenytoin at the silica surface can be strongly altered. Similarly to the spin-cast process, the choice of the solvent has a decisive impact on the film properties and thus on the morphology after the solvent removal. Using acetone and low phenytoin concentrations results in structures with a strong 2D growth tendency. This shows that the growth is not as strongly favored along one direction compared to the other samples, which have a needle-like morphology. The rapid solvent removal means that molecules have to assemble quickly into a crystalline state which is most likely not only at low energetic crystal sites, but also just in the vicinity and structures without sharply defined morphologies. At higher API concentrations, the crystals grow in a boomerang-type shape with both branches being nearly identical. In addition, adjacent boomerangs deviate in their size whereby on the one site larger and on the other site smaller boomerangs are present. Similar to other samples vacancies in the crystallites exist. As the amount of phenytoin is higher, an arrangement of the molecules on lower energetic crystal sites is more likely, and the two arms grow nearly identical. Further the boomerang arranges along defined directions which means that a long-range order exists in the areas shown in Figure 5c. This is quite surprising as some of these structures are not connected. In addition, the silica surface is isotropic, which means that a defined growth direction must be a consequence from something different from the substrate.

Comparison of the boomerang shape with the results from a 1.0 wt % EtOH solution and fast evaporation shows some similarities. The crystals observed for the slow processing in EtOH reveals fishbone-type structures, meaning that the crystals branch in the backward direction as the crystals grow in the forward direction. From a common center, this structure replicates three times with each inclining by 45°, 140°, or 180° with respect to the first direction. This shows that all directions are equally likely to develop. While the 180° direction is a result of symmetrical considerations, i.e., the structure is mirrored along a common center, the other directions are already present in the center of the structure that have a rectangular shape. From these structures the fishbone branches are initiated and grow for several hundreds of micrometers along the surface. Equally, to the acetone processed structures, the EtOH processed ones have a preferred 001 texture.

For the acetone sample with its boomerang shapes, a similar growth is proposed meaning that the fishbone- and boomerang-type structures are a result of the same mechanism. However, as the solvent evaporates, some of the interconnections of adjacent boomerangs are disrupted. From the experiments it cannot be decided if this takes place in an early stage or later stage of the solvent evaporation or if it is a result of rearrangements happening after solvent removal in the dry state. However, as the molecules have to diffuse a fair distance, it is more likely that this breaking of the interconnection takes place still while the solvent is in place; a solvent typically assists in API diffusion.

Using EtOH as processing agent with low phenytoin content and slow evaporation speeds results in the formation of flat, elongated structures. As the system has now more time for the assembling, a molecular adsorption at low energetic crystal sites take place. In the case of phenytoin this is at the head of the needle. At some points preferable growth along other directions

are noticed, forming side branches that however grow after a short distance in a similar direction.

At a high phenytoin concentration in EtOH, needle-like structures are present. At fast evaporation speeds the needles have a common center. At lower evaporation speeds, more extended needles are present, which have interconnection points but do not have common centers. The solubility of phenytoin in EtOH is about 1.8 wt % at room temperature, which means that at a concentration of 2.0 wt % the solution is already slightly supersaturated. This results in fast nucleation and crystal growth. As the growth takes place at a point at which a lot of residual EtOH is present, molecular diffusion is fast and growth along favorable sites takes place. Such a side is the head or tail of the needle as mentioned earlier. Anyway, a faster evaporation induced crystallization originates at certain points from which many crystals grow but all directions are equally likely present; thus all crystals have a similar size. On the other site, slow removal results in a larger spread of the crystal size as each crystal is initiated at slightly different times, and thus the Ostwald rule of stages influence the forming structures markedly; a larger crystal has a stronger tendency to increase its size compared to a smaller one.<sup>33</sup> In addition, some of the needles show intersection points with one needle being on top of another. This suggests that the crystal growth already takes place in the bulk solution, meaning that the structures form independent of the surface in use. This fact is supported by X-ray diffraction experiments that do not exhibit a preferred orientation. The absence of net planes perpendicular to the long needle axis is a result from the long needles adapting a flat confinement on the solvent removal or in other words just fall flat onto the surface.

The slowest crystallization is observed for samples prepared from physical vacuum deposition which initially results in amorphous films. The relatively high density together with the absence of a solvent results in the diffusion being limited and slow. Depending on the initial layer thickness, this results in long elongated needle-like structures that are more or less densely packed. Within the thinner sample, a disadvantage of the phenytoin molecules with the silica surface exists which on the other hand favors molecular movement of the API molecules along the substrate surface. This leads to an assembling on favorable crystalline sites. Similar to the other samples, this is on the head or tail of a needle. In some regions of the film a more spherulite-like structure develops as most likely the mobility of the molecules is reduced. The reduction of the mobility may be a result of local deviations of the silica substrate surface properties or a different nuclei being present as the spherulitic center. A nucleus which has a different orientation with respect to the surface also induces other facets being at the growth front. Thus, a distinct structure results.

The amorphous film was investigated 24 h after the preparation. Besides the crystalline structures being visible in Figure 7a also amorphous drops can be identified. These drops reflect the strong dewetting tendency of the API molecules from the interface. The remaining amorphous structures means that the time frame was not sufficient to crystallize all of the material. As mentioned earlier the crystallization may be enhanced by applying other preparation procedures. Anyway, increasing the layer thickness of the amorphous film results in the crystalline morphology being different and densely packed structures are noted. Typically, a thicker film means that the interaction with the substrate is of less importance suggesting that the amorphous bulk above the surface can stabilize its own

amorphous state longer. Many long needle-type structures develop from a single point within the phenytoin thin film typical for spherulitic growth. The experiments do not allow one to determine if the crystallization is initiated at the solid–solid, solid–air, or within the bulk. However, the homogeneous crystallization may also indicate that crystallization is initiated at the solid–air interface which can be rationalized by the fact that the rearrangement of the molecules at this interface is more easily accessible.

The investigation shows that all morphologies are a result of one polymorphic structure. This is surprising as often a strong change in the morphology shows a deviating internal arrangement or packing.<sup>34</sup> Using different processing procedures or sample treatments (heat, solvent annealing, among others) may allow tuning of the internal structure. Anyway, the fabrication techniques used in this study and the resulting isomorphic behavior make this system a perfect candidate for further investigations in terms of growth behavior and dissolution behavior.

## ■ CONCLUSION

The different preparation procedures shown in this work allow tuning the crystal morphology of phenytoin. While most of the samples show a preferred 001 texture with respect to the surface some of the samples reveal a powder-like character but with all samples crystallizing in one unique polymorph. This shows that the type of preparation has a decisive impact on the substrate interaction; i.e., for instance, low-concentrated EtOH solution results in an adaption of the phenytoin molecules with respect to the silica surface, while higher concentrations show a powder-like character typically for crystal formation in the bulk solution. Similarly, the formation of the various morphologies also indicates an altered dissolution behavior due to the differences in accessibility, i.e., the time dependent dissolving, of the distinct polymorphs varies. In general, the presence of different dominating crystal facets within a sample means also a variation in the dissolution behavior. This is due to the surface energy of each facet being different which causes the interaction of the solvent being favored or disfavored. From the measurements it can be concluded that the applied techniques allow to gain a lot of information on the formation of different crystal morphologies which is also of great interest within other research areas. Surprisingly the investigation of phenytoin reveals only one polymorph being present for all of the samples, which is expected to be different for other APIs or organic molecules.

## ■ ASSOCIATED CONTENT

### Supporting Information

Grazing incidence diffraction data, specular scans of the amorphous films and power spectral density patterns of the spin coat samples. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*E-mail: oliver.werzer@uni-graz.at.

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This research was funded by the Austrian Science Fund (FWF) [P25541-N19].

## REFERENCES

- (1) Fasano, A. Modulation of intestinal permeability: an innovative method of oral drug delivery for the treatment of inherited and acquired human diseases. *Mol. Genet. Genomics* **1998**, *64* (1), 12–18.
- (2) Sansone, F.; Barbosa, S.; Casnati, A.; Sciotto, D.; Ungaro, R. A new chiral rigid conewater soluble peptidocalix [4] arene and its inclusion complexes with  $\alpha$ -amino acids and aromatic ammonium cations. *Tetrahedron Lett.* **1999**, *40* (25), 4741–4744.
- (3) Neslihan Gursoy, R.; Benita, S. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomed. Pharmacother.* **2004**, *58* (3), 173–182.
- (4) Pouton, C. W. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and ‘self-microemulsifying’-drug delivery systems. *Eur. J. Pharm. Sci.* **2000**, *11*, S93–S98.
- (5) Leuner, C.; Dressman, J. Improving drug solubility for oral delivery using solid dispersions. *Eur. J. Pharm. Biopharm.* **2000**, *50* (1), 47–60.
- (6) Schrank, S.; Kann, B.; Saurugger, E.; Ehmann, H.; Werzer, O.; Windbergs, M.; Glasser, B. J.; Zimmer, A.; Khinast, J.; Roblegg, E. Impact of Drying on Solid State Modifications and Drug Distribution in Ibuprofen-Loaded Calcium Stearate Pellets. *Mol. Pharmaceutics* **2014**, *11*, 599–609.
- (7) Pouton, C. W. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. *Eur. J. Pharm. Sci.* **2006**, *29* (3), 278–287.
- (8) Rabinow, B. E. Nanosuspensions in drug delivery. *Nat. Rev. Drug Discovery* **2004**, *3* (9), 785–796.
- (9) Khinast, J.; Baumgartner, R.; Roblegg, E. Nano-extrusion: a one-step process for manufacturing of solid nanoparticle formulations directly from the liquid phase. *AAPS PharmSciTech* **2013**, *14* (2), 601–604.
- (10) Werzer, O.; Baumgartner, R.; Zawodzki, M.; Roblegg, E. Particular Film Formation of Phenytoin at Silica Surfaces. *Mol. Pharmaceutics* **2014**, *11*, 610–616.
- (11) Nokhodchi, A.; Bolourchian, N.; Dinarvand, R. Crystal modification of phenytoin using different solvents and crystallization conditions. *Int. J. Pharm.* **2003**, *250* (1), 85–97.
- (12) Singhal, D.; Curatolo, W. Drug polymorphism and dosage form design: a practical perspective. *Adv. Drug Delivery Rev.* **2004**, *56* (3), 335–347.
- (13) Kayaert, P.; Van den Mooter, G. Is the amorphous fraction of a dried nanosuspension caused by milling or by drying? A case study with Naproxen and Cinnarizine. *Eur. J. Pharm. Biopharm.* **2012**, *81* (3), 650–656.
- (14) Van Eerdenbrugh, B.; Van den Mooter, G.; Augustijns, P. Top-down production of drug nanocrystals: nanosuspension stabilization, miniaturization and transformation into solid products. *Int. J. Pharm.* **2008**, *364* (1), 64–75.
- (15) Liversidge, G. G.; Cundy, K. C. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. *Int. J. Pharm.* **1995**, *125* (1), 91–97.
- (16) Kipp, J. The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs. *Int. J. Pharm.* **2004**, *284* (1), 109–122.
- (17) Anders, R.; Merkle, H. P. Evaluation of laminated muco-adhesive patches for buccal drug delivery. *Int. J. Pharm.* **1989**, *49* (3), 231–240.
- (18) Lavan, D. A.; McGuire, T.; Langer, R. Small-scale systems for in vivo drug delivery. *Nat. Biotechnol.* **2003**, *21* (10), 1184–1191.
- (19) Werzer, O.; Kunert, B.; Roblegg, E.; Zimmer, A.; Oehzelt, M.; Resel, R. Surface Induced Order of Solution Processed Caffeine Needles on Silica and Muscovite Mica. *Cryst. Growth Des.* **2013**, *13*, 1322–1328.
- (20) Werzer, O.; Stadlober, B.; Haase, A.; Oehzelt, M.; Resel, R. Full X-ray pattern analysis of vacuum deposited pentacene thin films. *Eur. Phys. J. B* **2008**, *66*, 455–459.
- (21) Werzer, O.; Porzio, W.; Trimmel, G.; Plank, H.; Resel, R. Biaxially aligned crystallites of a fluorene-bithiophene co-polymer. *Eur. Polym. J.* **2013**, *49* (1), 177–183.
- (22) Werzer, O.; Resel, R.; Chernev, B.; Plank, H.; Rothmann, M. M.; Strohriegl, P.; Trimmel, G.; Rapallo, A.; Porzio, W. Crystallographic structure and morphology of bithiophene-fluorene polymer nanocrystals. *Polymer* **2011**, *52* (15), 3368–3373.
- (23) Diao, Y.; Myerson, A. S.; Hatton, T. A.; Trout, B. L. Surface design for controlled crystallization: The role of surface chemistry and nanoscale pores in heterogeneous nucleation. *Langmuir* **2011**, *27* (9), 5324–5334.
- (24) Ehmann, H. M.; Werzer, O. Surface mediated structures: stabilization of metastable polymorphs on the example of paracetamol. *Cryst. Growth Des.* **2014**, *14*, 3680–3684.
- (25) Ehmann, H. M.; Kellner, T.; Werzer, O. Non-contact-mode AFM induced versus spontaneous formed phenytoin crystals: the effect of layer thickness. *CrystEngComm* **2014**, *16*, 4950–4954.
- (26) Birkholz, M. *Thin Film Analysis by X-ray Scattering*; Wiley-VCH: Weinheim; 2006.
- (27) Nečas, D.; Klapetek, P. Gwyddion: an open-source software for SPM data analysis. *Cent Eur. J. Phys.* **2012**, *10* (1), 181–188.
- (28) Camerman, A.; Camerman, N. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1971**, *27*, 2205–2211.
- (29) Ehmann, H. M.; Baumgartner, R.; Kunert, B.; Zimmer, A.; Roblegg, E.; Werzer, O. Morphologies of Phenytoin Crystals at Silica Model Surfaces: Vapor Annealing vs. Drop Casting. *J. Phys. Chem. C* **2014**, *118*, 12855–12861.
- (30) Ehmann, H. M.; Zimmer, A.; Roblegg, E.; Werzer, O. Morphologies in Solvent-Annealed Clotrimazole Thin Films Explained by Hansen-Solubility Parameters. *Cryst. Growth Des.* **2014**, *14* (3), 1386–1391.
- (31) DDBST; <http://ddbonline.ddbst.de/AntoineCalculation/AntoineCalculationCGI.exe?component=Ethanol> (accessed 10.10.2013).
- (32) Noyes, A. A.; Whitney, W. R. The rate of solution of solid substances in their own solutions. *J. Am. Chem. Soc.* **1897**, *19* (12), 930–934.
- (33) Nývlt, J. The Ostwald rule of stages. *Cryst. Res. Technol.* **1995**, *30* (4), 443–449.
- (34) Wedl, B.; Resel, R.; Leising, G.; Kunert, B.; Salzmann, I.; Oehzelt, M.; Koch, N.; Vollmer, A.; Duhm, S.; Werzer, O.; Gbabode, G.; Sferrazza, M.; Geerts, Y. Crystallisation kinetics in thin films of dihexyl-terthiophene: the appearance of polymorphic phases. *RSC Adv.* **2012**, *2* (10), 4404–4414.