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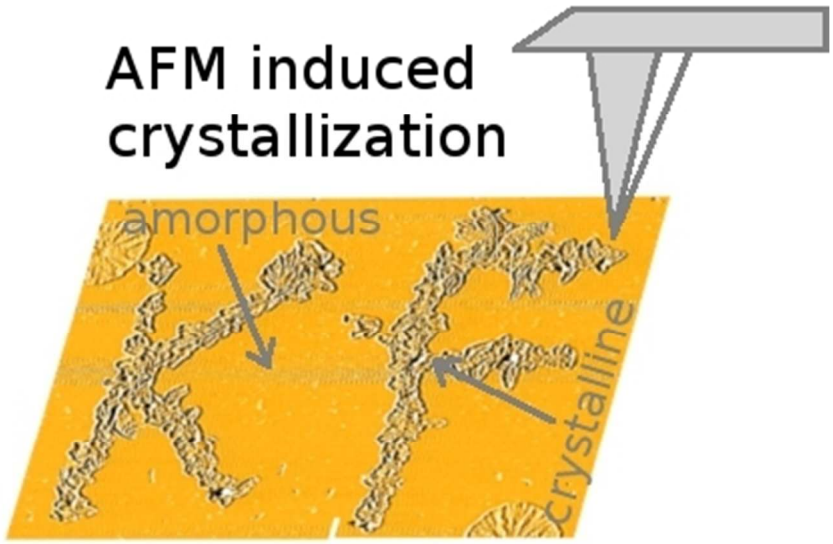

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Non – contact - mode AFM induced versus spontaneous formed phenytoin crystals: the effect of layer thickness

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In this work the model substance phenytoin is vacuum deposited onto a silica substrate resulting in amorphous films. Crystallization is obtained via the AFM tip as it moves in close vicinity of the surface in non – contact mode. The formed crystals show strong differences depending on the initial layer thickness and in comparison with spontaneously formed crystals.

The preparation of films with defined morphologies and crystallographic structures is of high interest in a variety of fields including pharmaceutical science,[1-3] colloid and surface science,[4-6] organic electronics[7-9] amongst many more. Within pharmaceutics, defined and reproducible film production is required to guarantee uniform dosage application and dissolution properties.

The solubility of a certain material strongly depends on its crystalline structure[10] and the morphology.[2, 10] While amorphous states exhibit favourable dissolution behaviour such films lack in long time stability on storage[11] thus stable crystalline structures are preferable. Often crystallization from an amorphous bulk is time consuming which increases processing times and costs. Small particular crystallites increase the maximum accessible solubility and reduce the time required for dissolution significantly[2] which both enhance the therapeutic action and reduce the mass load required to achieve a therapeutic *in-vivo* dose.

Defined crystalline structures can be obtained via various methods including spin-coating,[12] drop casting,[12] dip coating,[12] physical vapour deposition,[13, 14] vapour annealing,[15] with each providing advantages for certain classes of materials. For instance, preparing thin films of phenytoin via a spin cast process results in the formation of layer consisting of small, spontaneously formed crystalline particles which have favourable dissolution properties.[2]

Another highly interesting approach is the usage of mechanical stress to enhance crystallization which fastens the crystallization.[16, 17] Using an atomic force microscopy tip for the stress induction even allows for defined crystallization at defined spots which can be used to prepare highly structured surfaces.[18, 19] Typically the induction of crystallization is a result of the AFM tip being in contact with the surface. However, in our work we demonstrate the usage of non – contact mode AFM measurements for inducing defined crystallization within amorphous phenytoin thin films. Phenytoin is typically used due to its anticonvulsive, antiepileptic and anti-arrhythmic properties within therapeutic treatments of humans. Within this study the molecule is chosen as high quality amorphous films can be prepared which together with its isomorphic structure makes it a perfect candidate. The work will demonstrate how the initial layer thickness of the amorphous film influences the crystal morphology forming during the AFM treatment. For sake of comparison the results are benchmarked to spontaneously formed crystals.

5,5-Diphenylhydantoin (phenytoin) was purchased from Sigma – Aldrich, Germany) and used without further treatment. The substrates consisted of single crystal silicon wafers (Siegert Wafers, Germany) which had a thermal growth oxide of 150 nm. The wafers were cut into pieces and cleaned in acetone, isopropanol and NaOH solutions and dried under a nitrogen stream prior the experiments. Vacuum deposition of phenytoin was performed in a custom made vacuum chamber (10^{-4} mbar). The sample was kept at room temperature while the sublimation temperature was 110°. This provided a deposition rate of 1 nm per minute which was checked with the atomic force microscope. The AFM induced crystallization was performed with an Easyscan 2.0 AFM microscope (Nanosurf, Switzerland) via non-contact scans of various scan ranges. The setpoint was uniquely set to 50%. The cantilever used for the experiments were non – contact Tap-190 (Budgetsenors, Romania) with a resonance frequency of 190 kHz and a spring constant of 48 N/m. The scan rate for all measurements was 8 minutes per image. All experiments were performed under ambient conditions. Image processing was performed with the software package Gwyddion.[20]

The vacuum deposition of phenytoin at a silica surface results in the formation of amorphous films at a solid surface. The layer thickness can be easily varied by changing the deposition time. At short deposition time a coverage of about 25% phenytoin is obtained with the material assembling in a drop like structure at the silica surface as it can be seen by the non – contact mode AFM height image (Figure 1a). Droplet formation is typical for dewetting, i.e. phenytoin tries to minimize its contact with the silica surface on account of their difference in the hydrophilicity;[21] silica is hydrophilic with a water contact angle of about 35° while phenytoin is hydrophobic with a contact angle of 85°. The AFM measurement

reveals a homogenous distribution of these droplets with a mean diameter of 130 nm and a mean height of 30 nm. A fine structure within the droplets is not present which strongly indicates that the droplets are purely amorphous.

During the first AFM scan in the upward direction only the drop like structures are observed. However as the AFM tip measures along the surface a second time in non – contact mode the drop like structures have disappeared but instead small rod like crystalline structures are present (see Figure 1b). Repeated measurements on the same spot did not show any change of these crystalline structures. This reveals that the first measurement was sufficient to achieve a stable crystalline structure at the silica surface which were induced by the AFM tip. The crystallites show a mean grain size of 380 nm and a mean grain height of about 70 nm. Comparing these values with those of the initial drop like structure shows that the crystallites are more extended and, consequently, the number of crystallites is smaller than the number of the initial droplets. As the interaction of the phenytoin with the silica surface is weak molecular diffusion along the surface is fast. Thus crystalline fractions attract amorphous molecules along the surface from adjacent drops and incorporate them into an already formed crystal which is in agreement with Ostwald - ripening.[22] As a result individual crystals are larger than the initial droplets.

Typically AFM – induced crystallization is performed by contact mode measurements, i.e. the tip is in contact with the surface as it slides along[19, 23] or is indented[24]. It is reported that the AFM tip causes a perturbation which induced nucleation by reducing the nucleation barrier.[18] However, in this work non – contact measurements are used.[25] This means that the silicon tip is in the vicinity of the surface (~20 nm separation) but hardly has contact. Any interaction therefore must be a results from short range forces which in air are dominated by attractive van der Waals interactions. Such forces cause a perturbation by pulling the amorphous parts toward the cantilever. This seems to be sufficient to achieve nucleation within the amorphous phenytoin film followed by crystal growth. An amorphous phase typically means that phenytoin is in a supersaturated state which requires only a small disturbance to transfer into a crystalline state and thus a droplet rapidly crystallizes.

Measurements over a time frame of 8 h did not show a significant change of the crystallization behaviour meaning that the amorphous droplets remain stable until an AFM measurement is performed. This indicates that the silica surface is able to stabilize the amorphous film. However after a time frame of 24 hours the situation changes and the presence of spontaneously formed crystallites can be observed (see Figure 1c). These crystallites cover nearly the entire surface, but in some parts of the surface drop like structure are still noticeable (see right down corner in Fig. 1c) showing that after a time frame of 24 h still some phenytoin remains in the amorphous state. Comparing these formed structures with the AFM - induced crystallites reveals differences. On the first view, the film consists of small crystallites distributed across the surface. On a more detailed inspection, it can be noted that the small crystallites are interconnected at one end. In addition the spontaneously crystallized phenytoin exhibits long range order within the scan range investigated. At the lower middle of the left hand side within Fig. 1c a center can be identified at which all the elongated structures come together. This is typical for spherulite type growth where nucleation is initiated at a spot within the surface. On account of the large surface coverage of the spontaneously formed crystal structures the heights of these are much lower compared to the previous sample and is on average just 7 nm. Comparing the morphology of the AFM – induced with the spontaneously formed crystals reveals that much more separated crystals form during the AFM measurements; as the tip is dragged slightly above the surface many interaction takes place. This permanent perturbation enhanced the probability for a crystallization initiation and more crystals form compared to the sample crystallized over time for which external perturbation is absent.

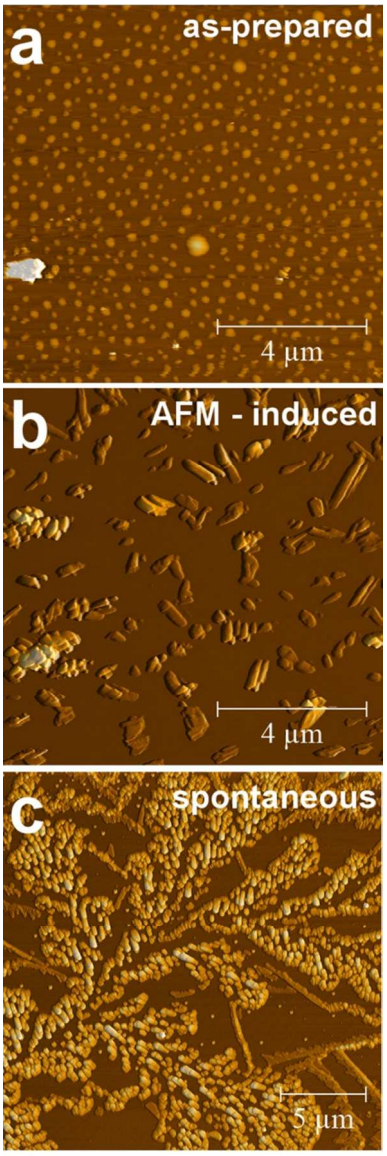

Figure 1: Phenytoin thin film with a sub monolayer coverage after the preparation process (a), after the AFM induced crystallization (b) and the spontaneously formed crystals.

The deposition of larger quantities of phenytoin onto the silica surface results in the disappearance of the drop like structure and a homogenous film forms. At a layer thickness of 15 nm this film nearly covers the entire surface but with some holes still remaining (see Figure 2a). During the first AFM measurements most of the film consists of amorphous phenytoin with some small crystallites are already noticeable. This shows that the crystallization is very rapid within thicker films and that a small disturbance from the AFM tip is sufficient to initiate the growth already at the first scan. This proofs the assumption that the silica substrate somehow stabilizes the amorphous state of surface near phenytoin. As the film thickness increases the substrate is no longer capable of stabilizing the amorphous state and crystallization is induced much more easily. At the left top end of the image a large crystal is eminent. The extension of this crystal suggests however that it had already formed during the

preparation process. Most likely a defect or some dust particle at the surface caused this crystal to grow directly after deposition. On the second measurement the film morphology further transfers from an amorphous appearance into needle like crystallites (see Figure 2b). The number and size of the crystallites is strongly increased compared to the first measurement. Repeated scans reveal that further crystallization takes place as additional measurements are performed. After five measurements nearly all of the material is transferred into crystallites with some small areas remaining in the amorphous state (flat areas in Fig 2c). Surprisingly the shape and size of the "defect" crystal already present in the first scan does not change which shows that an already formed crystal is not sensitive on the AFM measurement, i.e. additional molecules do not diffuse to the crystal in the time frame of the experiment, i.e. 40 minutes.

A comparison with the drop like film shows that a full crystallization of the amorphous film takes longer for the thicker film. The higher mass being present means that more material needs to diffuse for crystallization. In addition, this 15 nm thick phenytoin film covers the entire surface which means diffusion must take place at the phenytoin – air interface or within the bulk. Both are, due to cohesive interactions and sterical constrains, limited and the time required for the diffusion process increases, thus crystallization of the film requires more time.

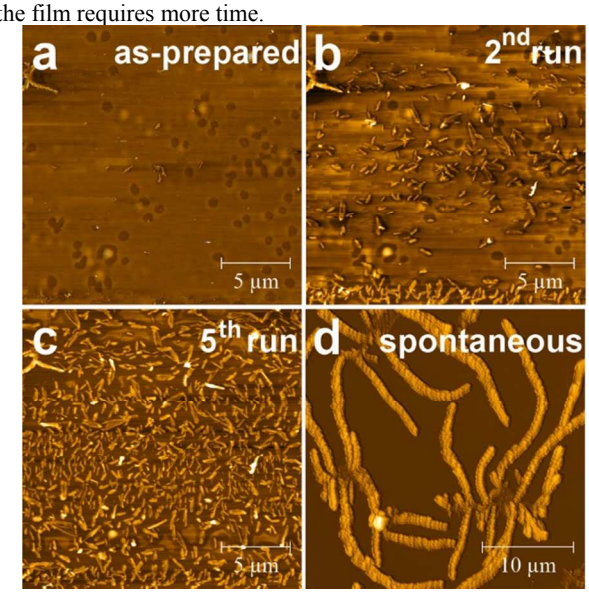

Figure 2: Phenytoin thin film with 15 nm thickness after the preparation process (a), the 2nd AFM scan (b), the 5th AFM scan induced (c) and the spontaneously formed crystals.

The spontaneous crystallization of phenytoin within the 15 nm thick film results in a morphology that is completely different compared to the previous investigated sample. Extended rod like structures of several tenth of µm length are visible (see Figure 2d). A height of 150 nm and a width of about 1 µm is determined. The height values are significantly larger compared to the spontaneously formed crystals of the thinner film which showed just 7 nm extension from the surface. This means that phenytoin diffuses to an initial crystallite over a long distance. As there is a densely packed amorphous bulk present, diffusion along the surface – air interface is more likely. The crystals grow therefore from the surface and a strongly 3 dimensional growth is present and as a result high and broad crystals appear. Similar to the thinner film, nucleation appears to be initiated at single spots along the surface and interconnection points are most likely the position where crystallization starts to develop. The similarity in the heights of the individual needles suggests that the formation of one crystal is not favoured in account of another.

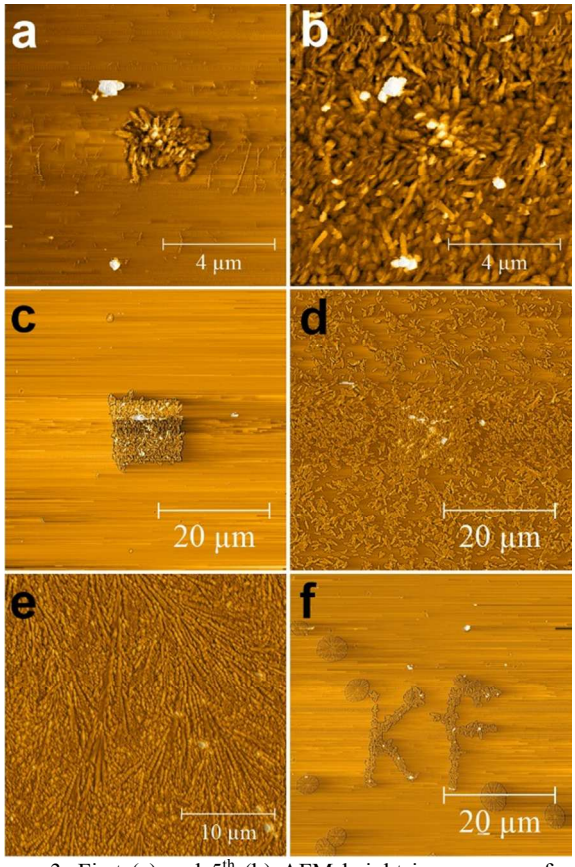

Figure 3: First (a) and 5th (b) AFM height image scan of a 50 nm thick phenytoin film. The first (c) and 5th (d) scan of the same sample at larger scan ranges. Spontaneously formed (e) and lithographically (f) written crystalline phenytoin surfaces.

Preparing a 50 nm thick amorphous phenytoin film results in a homogenous film with holes or droplets being absent. Similarly to the previous samples AFM induced crystallization can be induced. In Figure 3 a-d the AFM induced crystallization of the 50 nm film is illustrated by various scans and scan ranges. A 1 x 1µm^2 square was "written" via non-contact scans into the phenytoin film prior the 10 x 10 µm^2 measurement depicted in Fig. 3a. Surprisingly, the square is expended to a more bar like structure, thus the crystal growth overshoots the scan range on this small scale. This also indicates the resolution limit of structures that can be "written" into a surface of phenytoin. Repeated measurements at the same scan range exhibit crystal formation over the whole scan range and after the 5th measurements the scan range is fully covered with crystals (Fig. 3b). The root mean square roughness increased from initially 19 nm to 28 nm which shows that the crystalline film is more jagged compared with the amorphous film; the crystallites results in vacancies in the surface region which is represented by the rms value. The elongated crystals do not show any preferred orientation meaning that the AFM tip does induce crystallization but without imposing defined orientations. In principle the size of the "written" square can be increased a couple of times. This is indicated in Fig. 3c and 3d which shows the previous 10x10 µm^2 square measured at 50x50 µm^2 scan range. During the first measurement the square is clearly visible while after the 5th run the square is surrounded by various AFM – induced crystallites.

The spontaneously formed crystals of phenytoin developed in the 50 nm thick film is shown in Figure 3e. The crystalline structure

consists of rod like structures which originate from a common center, thus a spherulite type growth with small spherical side branches is again present. Other than in the thinner samples, this spherulite like structures cover the entire surface. The amount of phenytoin at the surface is high which results in densely packed branches which closely pack next to each other leaving no gaps within the crystalline film.

In Figure 3f the ability to use the AFM – induced crystallization for lithography is demonstrated. The letters K and F were generated by one time scans over the surface of a 100 nm thick phenytoin thin film. A subsequent measurements on a larger area exhibit the written structure. Some spontaneously formed spherulite crystals are also noted which are due to defects introduced during the preparation of the sample.

The various samples reveal the formation of crystalline structures spontaneously or AFM induced. As phenytoin is known to be isomorphic, i.e. only one crystal structures is known[10] it is very likely that all crystallites have the same structure. Variation in the contact plane of the crystals with the substrate may exist like shown elsewhere but was of no interest within this study.

In literature there are various examples of using contact mode AFM lithography.[26] As the AFM tip scratches along the surface pattering or crystallization can be induced within films containing organic molecules[18, 19] or polymers.[27] A direct comparison of the non – contact mode used in this work and contact mode crystallization can hardly be given as measurements on the same samples have to be performed. Anyway, it can be expected that the usage of either of these two methods provided advantages. For instance, for "stable" amorphous a strong disturbance may be required which favours the usage of contact mode which allows for stronger interact with the film. Variation of the cantilever materials allow to adjust the interaction strength. In the case of materials which have a strong adhesive tendency for the cantilever, like often present for polymers, a non – contact mode would be advantageous as the tip is not affected by bridging polymer chains. In any case, a comparison of the various methods on the very same samples have to be made to decide on the preferable method for the desired application.

Conclusion

In conclusion it can be stated that amorphous phenytoin transfer either by spontaneous crystallization or the interaction with the AFM tip into a stable crystalline form. Variation of the morphologies exists between this two approaches whereby the layer thickness has a strong impact. At very low coverage phenytoin is able to diffuse freely along the surface resulting in large crystalline structures. The number of crystallites can however be manipulated using the AFM which results in a strong increase in the number of crystallites preferable in pharmaceutical application. While the upscale for a high quantity use will be difficult this technique may give an insight in the understanding of particle – amorphous film interaction often being present in standard preparation techniques like fluidized bed or others.

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Notes and references

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