Stabilization of the Metastable Form I of Piracetam by Crystallization on Silicon Oxide Surfaces

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Supporting Information

ABSTRACT: The crystallization of the nootropic drug piracetam in spin-coated thin layers on silicon oxide surfaces was analyzed by optical microscopy, as well as powder and synchrotron grazing-incidence X-ray diffraction. We observed the crystal structure formed to depend on the concentration of the solution used in the preparation of the samples and identified either the polymorphic form I (for concentrations <0.015 mol·dm⁻³) or form II (for >0.147 mol·dm⁻³). Although significant dewetting of the materials occurred over time, the transformation of form I, which is metastable at room temperature, to the more thermodynamically stable forms II or III was not observed, even at the long-term (9 months). Even when subject to prolonged solvent-vapor annealing, dewetting but no alteration of the polymorphic form occurred. Our results suggest that the metastable piracetam form I is stabilized upon crystallization from solution in thin films, which might generally pave the way to stabilizing metastable forms of drugs of increased dissolution rate.

INTRODUCTION

One of the major challenges for the pharmaceutical industry is that many of the developed active pharmaceutical ingredients (APIs) present poor water solubility, which leads to a poor bioavailability of the drugs. This severe problem has created a high demand for new strategies to improve drug solubility. Some of the most studied approaches involve the preparation of solid dispersions, co-crystals, hydrates, or the inclusion of APIs present poor water solubility, which leads to a poor bioavailability of the drugs. This severe problem has created a high demand for new strategies to improve drug solubility. Some of the most studied approaches involve the preparation of solid dispersions, co-crystals, hydrates, or the inclusion of the API into nanoparticles. Likewise, modifying the particle size of the drug (thus increasing the surface area) or its crystal structure are possible strategies to the increase dissolution rate.

In the solid state, it is common that organic molecules crystallize in different crystalline structures, a phenomenon known as polymorphism. Different polymorphs exhibit distinct properties such as color, melting point, or solubility, which is due to different intermolecular interactions. Therefore, different polymorphs should be regarded, in fact, as different materials. Thus, controlling polymorphism can be employed as tool for tuning the properties of a drug to maximize its solubility and, as a result, its bioavailability. Such an approach, however, must be handled with care, as in the absence of kinetic barriers all polymorphs typically tend to evolve toward their most thermodynamically stable form, therefore altering the properties of the pharmaceutical product over time.

Recently, the formulation of drugs as thin films has gained attention as an alternative for improving bioavailability. This method has the advantage of increasing the surface area of the material and thus its dissolution rate. Moreover, due to constraints brought about by the limited thickness of the adsorbate and by its interaction with the substrate, thin-film growth of drugs has further the potential of stabilizing metastable phases or even new polymorphs, known as substrate-induced phases (SIPs). Because SIPs can, in fact, extend vertically over several molecular layers, they principally differ from self-assembled monolayers. Initially, SIPs have mostly been investigated in the field of organic semiconductors. This material class is typically based on aromatic molecules of high aspect ratio (sometimes also comprising alkyl side chains). In particular, prominent compounds like pentacene do not comprise functional or polar groups prone to inducing specific interactions with the substrate surface. Today, the occurrence of SIPs has been documented for a large variety of small molecular semiconductors. As is the case for polymorphism in bulk materials, a thorough study of the

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polymorphic behavior of a given compound is mandatory for thin films due to the well-known tendency toward SIP formation close to a substrate. The experimental conditions where each phase is obtained must be carefully determined in order to allow their selective production and storage. Likewise, their properties and stability domains, which can significantly vary from the phases obtained in the bulk, need to be evaluated in detail to this end.8,10

In the present work, the crystallization and polymorphic behavior of the nootropic drug piracetam, on silicon oxide surfaces, was studied. In principle, piracetam [2-(2-oxopyrrolidin-1-yl)acetamide, C₆H₁₀N₂O₂, CAS number 7491-74-9, Figure 1] is a relatively simple molecule with five known polymorphic forms. However, this compound comprises two polar groups and, therefore, a pronounced ability to form hydrogen bonds, which translates into complex intermolecular interactions if compared to typical organic semiconductors like pentacene, where dispersion and quadrupolar interactions dominate. As such, studying the polymorphism of polar compounds generally provides deeper insight into the crystallization of SIPs. Thermodynamic data for piracetam, found in literature, are summarized in the Supporting Information. Of the five known polymorphs of piracetam, forms IV and V are prepared under high pressure conditions (>0.5 GPa),21,22 while the remaining forms (I–III) can be obtained (under certain conditions) at room temperature and pressure.22–26 Thereof, forms II and III can be prepared by recrystallizing piracetam,22–26 and both forms can transform into form I upon heating beyond 400 K.22,23,25,27 Form I is metastable at room temperature but can be isolated, for a few hours, through rapid cooling before conversion into form II is observed.23,27 Interestingly, while form III is the most stable polymorph at room temperature,23,24,28–30 form II does not convert to form III in the absence of solvents that can trigger this transformation.24

**EXPERIMENTAL SECTION**

**Materials.** Piracetam (Sigma-Aldrich, >98%) and methanol (Chem-Lab, 99.8%) were used without further purification. The powder diffractogram of the piracetam starting material, recorded at room temperature, matched the calculated pattern for polymorphic form III of piracetam (Supporting Information, Figure S2). The data were indexed as monoclinic space group P2₁/n, a = 6.5326(19), b = 6.440(2), c = 16.463(5), and β = 92.19(3), in good agreement with the data reported in the literature.22–26 Thermogravimetric analysis (TGA) showed no mass loss of the compound up to Tₘ₂ = 525.90 K.32 DSC studies on the starting material support the thermodynamic relations between forms I, II, and III of piracetam, as previously reported in literature (Supporting Information).23–25 SiOₓ/Si(111) substrates (800 μm thickness, ca. 2 nm native oxide top layer) were cleaned using a UV/ozone (BioForce Nanosciences, Inc., Ames, IA) for 20 min. The substrate was repeatedly rinsed with methanol under a fast rotation program (1000 rpm for 5 s, 2000 rpm for 10 s, and 3000 rpm for 30 s) prior to the preparation of the samples.

**Methods.** The samples were studied by polarized optical microscopy (POM) using a Nikon Eclipse E200 microscope equipped with a digital Nikon DS-Fi1 camera and polarizing filters; images were treated using the NIS-Elements software package (version 3.0). Specular X-ray diffraction (s-XRD) and grazing-incidence wide-angle scattering (GIWAXS) patterns were obtained using a Rigaku Ultima IV diffractometer in out-of-plane and in-plane geometry, respectively. A Cu Kα radiation source was used with a tube amperage of 40 mA and voltage 40 kV. A step size of 0.02° and an acquisition time of 20 s/step was chosen for the scattering experiments. The indexation of the powder pattern was performed using the program Chekcell.33 Grazing incidence X-ray diffraction (GIXD) measurements were carried out at the KMC-2 beamline at the synchrotron light source BESSY II, HZB, Berlin.33 The measurements were performed at room temperature, with an incident angle of α = 0.2° (wavelength 1 Å), using an area detector (Bruker Vantec 2000). Data processing was carried out using the custom-made software package PyGid.34 In all diffraction data, the coordinates (qₓ and qᵧ) are the components of the scattering vector q, as defined by q = 4π/λ sin(θ), where λ is the wavelength of the radiation and θ is the scattering angle. Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer Pyris 6 TGA. DSC studies were performed on a Diamond DSC from PerkinElmer equipped with a PerkinElmer Diamond DSC autosampler. Polycrystalline powder of piracetam was obtained by recrystallization of the starting material in methanol by dissolving ~1 g of piracetam in 10 mL of solvent at room temperature. The solution was allowed to evaporate, and the precipitated crystals were separated by filtration, ground, and analyzed by X-ray diffraction. Thin-film samples were prepared by spin-coating of solutions of the starting material onto SiOₓ. Solutions of piracetam in methanol, with concentrations in the range 0.010–0.212 mol·dm⁻³, were used. A small volume of the solution was dropped on the substrate, and the spin-coating process was started. Two protocols (both composed of three steps with increasing speed) were employed. Method 2 used higher rotation speeds (500 rpm for 5 s, 1000 rpm for 10 s, 2000 rpm for 30 s) and a volume of 100 μL to ensure complete coverage of the substrate. Method 1 used lower rotation speeds (250 rpm for 5 s, 500 rpm for 10 s, and 1500 rpm for 30 s), with a volume of 50 μL of solution. Solvent vapor annealing (SVA) was performed to assess the stability of the samples. A glass chamber with a methanol-saturated atmosphere was prepared, and a previously characterized sample was placed inside for 5 days. Thereafter, the sample was removed and reanalyzed by POM, s-XRD, and GIWAXS.

**RESULTS AND DISCUSSION**

**Bulk Crystallization of Piracetam from Solution.** To juxtapose the crystallization process of the bulk material with that of the thin film, the starting material was crystallized from methanol through solvent evaporation. The crystals obtained were ground and analyzed by powder X-ray diffraction. Figure 2 compares the diffractogram with the calculated patterns of form II and III based on literature data.32 Peaks corresponding to both forms can be observed in the diffraction pattern, indicating that the crystallization of the sample resulted in a phase mixture between forms II and III. This result is in line with literature where piracetam is reported to crystallize first as form II and to transform over time to form III if the solvent is present.23,24

**Crystallization from Solution into Thin Films.** Spin-coating was used to prepare thin films of piracetam on silicon oxide substrates. This technique allows for the preparation of uniform thin films with thicknesses typically <1 μm. The sample thickness may be controlled by varying the concentration of the solution used in the spin-coating process.
The details of the preparation conditions are summarized in Supporting Information, Table S5 for all samples. The presence of piracetam crystals was not immediately evident after spin-coating when observed by microscopy. Within minutes, however, the crystallization of the film was observed, with nucleation occurring at the sample edges followed by the radial growth of the crystals, until complete coverage of the substrate was achieved (<2 h). Note that, normally, crystallization would originate from different points at the substrate with the formation of several domains, finally covering the substrate upon subsequent growth.

**Effect of Concentration of the Piracetam Solution.** Several samples were prepared from solutions of piracetam with increasing concentrations (between 0.010 mol·dm$^{-3}$ and 0.212 mol·dm$^{-3}$) to assess the effect of concentration on the sample morphology (using POM) and on the crystalline structure (by s-XRD and GIWAXS). In Figures 3 and 4, the results are shown for the most representative samples.

For all samples, the crystals exhibit the shape of long needles. In Figure 3b, it is apparent that if gaps do exist between the needles, crystal growth can occur perpendicularly to the needles to fill the gap. In some samples (from solutions of concentrations 0.041 mol·dm$^{-3}$ and 0.076 mol·dm$^{-3}$), we further observed the growth of long crystals on top of the needles (Supporting Information, Figure S7). From these long crystals, many smaller needle-shaped crystals appear to form with their growth perpendicular to the main needle.

The XRD results reveal that the crystal structure adopted in the samples sensitively depends on the concentration of the piracetam solution used (Figure 4). Samples prepared using solutions with $c = 0.015$ mol·dm$^{-3}$ and $c = 0.076$ mol·dm$^{-3}$), we further observed the growth of long crystals on top of the needles (Supporting Information, Figure S7). From these long crystals, many smaller needle-shaped crystals appear to form with their growth perpendicular to the main needle.

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characterized as above. One of the samples was stored and, thereafter, characterized again. The second was subject to SVA and afterward characterized. The evolution of the samples observed by POM is shown in Figure 6, and the respective XRD diffractograms is in the Supporting Information.

As shown in Figure 6a, before storing, the sample is composed of thin needles that are long (>200 μm) and thin (<4 μm), spreading out radially from nucleation centers. The crystals are stacked on the substrate and grow parallel to the surface. After nine months (Figure 6b), the crystals appear more individualized as well as significantly shorter (<100 μm), which indicates dewetting of the sample. This effect is particularly apparent at the domain boundaries, where empty spaces are observed. The XRD diffractogram obtained after the storage of the sample for nine months shows that the sample retained polymorphic form I over this period (Supporting Information, Figure S10). These results indicate that, despite the fact that form I is normally unstable at room temperature (typically converting to form II within a day),\textsuperscript{25,28,29} it can, indeed, be stabilized over a long period of time when crystallized in the form of thin layers.

It is clear from the presented POM data that SVA induces significant dewetting of the adsorbate (Figure 6c–e). The images obtained immediately after SVA show that in the sample individual agglomerates had formed. However, 1 h after removing the sample from the SVA chamber, recrystallization of these agglomerates into crystalline needles was observed.

The diffractograms revealed that the recrystallized sample obtained at the end of the annealing process consisted of form I (Figure S16), indicating that this form is, indeed, the stable polymorphic form upon piracetam crystallization on silicon oxide substrates (at low concentration).

The orientation of the samples in relation to the substrate can be deduced from our combined s-XRD and GIWAXS experiments (Figure 4): the peaks observed in Figure 4 indicate that if form I is formed, only the (120) diffraction peak occurs in s-XRD and (002) in GIWAXS (see Figure 7). When crystallization occurs into form II, the main peak in s-XRD is (002). As summarized in Figure 7, the texture of the samples also changes depending on the experimental conditions. As seen in the Supporting Information, after storage for nine months, only the peak for the form I (101) plane is observed. This indicates that a reorganization of the molecules in the...
sample occurred over time, however, without a change in the polymorphic phase. This is also observed when the samples are subject to SVA: immediately after preparation, the most prevalent peak is (120), after SVA for 5 days, however, this peak had disappeared, and the data is dominated by diffraction from the (110) lattice planes. Notably, this indicates that the (120) texture of piracetam is not most stable, and, if allowed to evolve, the orientation changes, while retaining the crystal form.

**CONCLUSIONS**

We present and document a method for reproducibly preparing crystals of the polymorphic forms I and II of piracetam by growing thin films on silicon oxide surfaces at room temperature under the variation of the concentration of the solution used in spin-coating. The results show that, depending on the concentration, spin-coating of piracetam leads to the formation of either form II (high concentrations) or of the metastable form I (low concentrations). The latter was observed to be stable on the substrate over a period of several months, although dewetting of the sample occurred over time. Likewise, SVA of form I samples leads to dewetting and subsequent recrystallization, however, again into form I. Our results further indicate that form I tends to grow in (120) texture, while form II grows in (002) texture. Form I tends to transform its orientation in the thin film over time into the (101) texture and upon SVA into the (110) texture.

Our findings can now be used as a selective and reproducible protocol for preparing samples composed of either form I or form II. These results point toward a new, metastable, and potentially more soluble formulation of the nootropic drug piracetam. Going beyond SiO$_2$ as model substrate, analogous studies for biocompatible substrates could pave the way toward...
enhancing the bioavailability of piracetam in pharmaceutical applications.

**ASSOCIATED CONTENT**

1. Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.cgd.8b00533.

Summary of the main structural and thermodynamic data for piracetam reported in the literature; phase diagrams for forms I–III of piracetam; X-ray powder diffraction of the piracetam powder sample compared to the calculated pattern for piracetam form III; corresponding indexation of the X-ray powder diffraction pattern; TGA thermogram of the piracetam powder; DSC studies performed on piracetam; preparation conditions of the thin film samples studied in this work. all POM images taken of the samples as a function of the concentration; schematic representation of the X-ray techniques used; XRD results for the samples as a function of concentration; s-XRD pattern of the silicon oxide wafer; GIXD measurements for the samples as a function of concentration; s-XRD results of sample 10 compared to the calculated main reflections piracetam form I powder (black lines); XRD diffractograms of samples 10 and 9, respectively (PDF).

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Notes

The authors declare no competing financial interest.

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**REFERENCES**


