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Manipulating the Crystalline State of Pharmaceuticals by Nanoconfinement

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ABSTRACT

We show that nanoconfinement is a handle to rationally produce and stabilize otherwise metastable or transient polymorphs of pharmaceuticals, as required for controllable and efficient drug delivery. The systematic investigation of crystallization under confinement unveils thermodynamic properties of metastable polymorphs not accessible otherwise and may enhance the understanding of the crystallization behavior of pharmaceuticals in general. As an example in this case, we studied acetaminophen confined to inexpensive and biocompatible nanoporous host systems. Calorimetric and X-ray scattering data clearly evidence that either the stable polymorph form I or the metastable polymorph form III can be stabilized in high yields. Thermodynamic parameters for form III of acetaminophen are reported, and strategies to manipulate the crystalline state in pores by thermal treatments are presented.

The manipulation of the crystalline state of polymorphic substances is an important issue in several fields of application.[1] Polymorphic pharmaceuticals confined to nanoporous host systems show different crystallization behavior than in the bulk.[2,3] This can be used to manipulate the crystalline state of pharmaceuticals and to optimize their properties.[4,5] Acetaminophen ($C_8H_9NO_2$, inset Figure 1), also known as paracetamol, is a common analgesic and antipyretic drug that exists in three different crystalline forms. The commercially used monoclinic form I is the most stable polymorph with a melting temperature of about 167−169 °C. The orthorhombic form II is metastable and melts at ≈156 °C. Several approaches have been reported to produce these crystalline forms from solution or from the melt.[6−9] A third polymorph, form III, is unstable in bulk systems and occurs only under specific conditions, for example, when acetaminophen is confined between glass plates or to glass capillaries.[6,10,11] Only a little is known about the physical properties of form III, even though RAMAN spectra[10] and wide-angle X-ray scattering (WAXS) data were reported.[11] Previous findings may indicate the occurrence of form III as a transient state in bulk acetaminophen. The conversion of spherulites obtained by cold crystallization to small stacked platelets near 120 °C[10] and a small exothermic dip in the same temperature range in differential scanning calorimetry (DSC) heating curves[12] were related to a bulk solid−solid transition from the transient form III to form II. However, melting of form III has never been reported, and its thermodynamic parameters are basically unknown. No viable method to intentionally produce form III in a controlled manner has been reported to date. Therefore, acetaminophen represents an ideal polymorphic model system to demonstrate the manipulation of the crystalline state of drugs by imposing nanoconfinement. As we will show, this strategy easily allows producing either the metastable form III or the thermody-

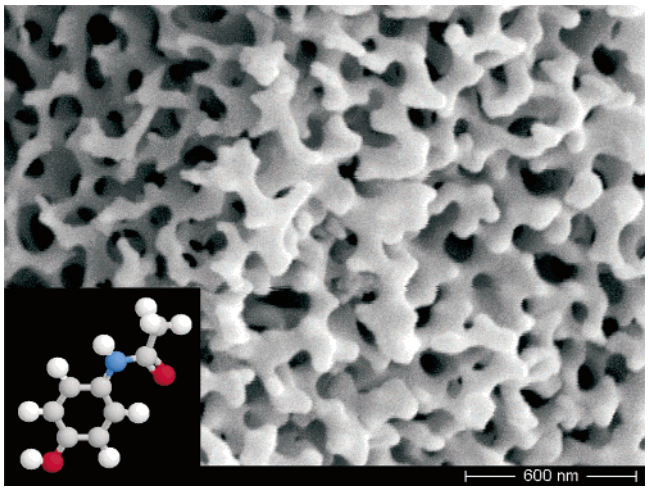

Figure 1. Scanning electron microscopy image of a controlled porous glass specimen. Inset: chemical structure of acetaminophen (gray: carbon; white: hydrogen; red: oxygen; blue: nitrogen).

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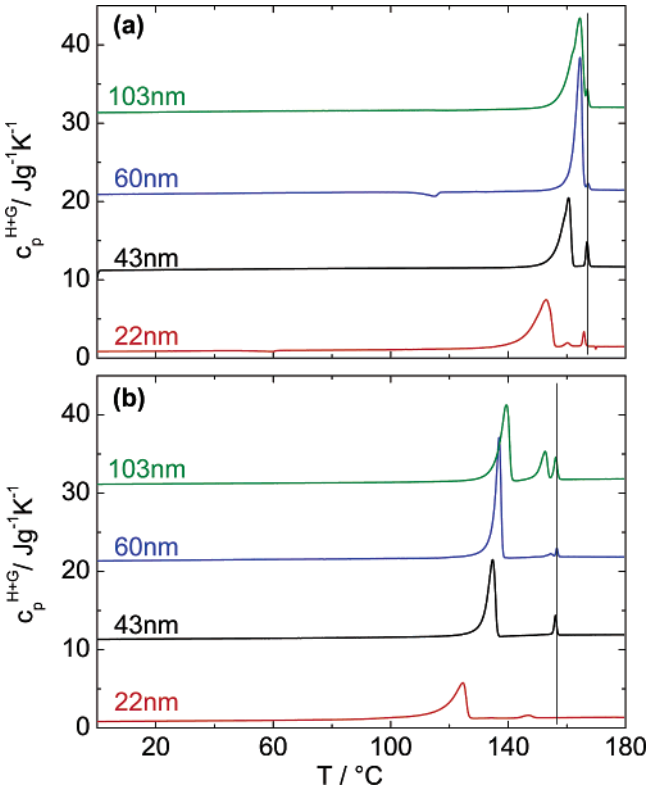

Figure 2. DSC heating scans (dT/dt = +10 K/min) on acetaminophen infiltrated into CPGs with different d values. The total heat capacity c_p^{H+G} of the host−guest systems is plotted. (a) Melting of form I crystallized during cooling from 180 °C to room temperature in the presence of a bulk acetaminophen reservoir on the surface of the specimens. (b) Melting of form III obtained by quenching and subsequent isothermal crystallization at 80 °C for 2 h after the removal of any bulk acetaminophen from the specimen surface. An additional peak at ≈152 °C occurring for d = 103 nm indicates melting of confined form II crystals. The lines at (a) $T_{M,I} \approx 167$ °C and (b) $T_{M,II} \approx 156$ °C denote melting peaks originating from residual acetaminophen on the surface of the specimens. The curves are vertically shifted by 10 J/gK (d = 43 nm), 20 J/gK (d = 60 nm), and 30 J/gK (d = 103 nm).

namically stable form I of acetaminophen in high yields as well as specific mixtures of different crystalline forms.

To impose nanoconfinement, we infiltrated acetaminophen into controlled porous glasses (CPGs, Figure 1).[13] The CPG specimens had a thickness of 300 μm, a porosity of about 50% , and a spongelike morphology. Their interconnected pores had well-defined diameters of 22, 43, 60, and 103 nm. We immersed CPGs into acetaminophen melts heated to 180 °C. After removing the samples from the melt, acetaminophen inside the pores crystallized during cooling while it was still connected to bulk acetaminophen on the surface of the specimens. We removed the latter carefully with scalpel to obtain samples predominantly containing acetaminophen confined to the nanopores of the CPGs and measured DSC heating scans (Figure 2a). Melting of form I of acetaminophen is observed for all samples but the maximum of the melting peak T_M shifts to significantly lower temperatures as the pore diameter d decreases. For acetaminophen confined to nanopores with the largest d value of 103 nm, the T_M value of 167 °C is only slightly below the bulk melting temperature of form I ($T_{M,I}(\infty)$ = 167−169 °C) while decreasing d to 22 nm shifts T_M to about 152 °C.

Using the same set of samples, the acetaminophen inside the CPGs was molten for 3 min at 180 °C and quenched at a nominal rate of −200 K/min to −40 °C without being connected to a bulk reservoir. Like in bulk acetaminophen,[9,12] this leads to nearly 100% amorphous, glassy acetaminophen, as obvious from DSC heating scans measured on quenched samples (not shown). Cold crystallization occurred upon heating the samples to temperatures significantly above the glass transition at 24 °C. DSC heating scans measured after isothermal crystallization at T_c = 80 °C for 2 h revealed that all samples were completely crystallized, independent of the d value (Figure 2b). This was deduced from a comparison of the total heats of melting Δh_M determined from the sets of heating scans seen in Figure 2a,b. However, the confined acetaminophen isothermally crystallized at T_c = 80 °C (Figure 2b) consistently melts at significantly lower T_M values than acetaminophen crystallized upon cooling in the presence of a bulk reservoir (Figure 2a). Also, a pronounced melting point depression can be seen in Figure 2b: T_M shifts from 139 °C for d = 103 nm to 125 °C for d = 22 nm. An additional peak at 152 °C in the heating scan of acetaminophen in CPGs with d = 103 nm indicates melting of form II under confinement, as discussed below.

Confinement-induced melting point depression is a well-known phenomenon[14−16] and can be thermodynamically described by assuming equivalence of the free energies of the confined liquid and crystalline phases at T_M. The melting temperature $T_M(d)$ of crystals confined to cylindrical pores can be calculated using a Gibbs−Thomson equation having the form[3,15]

$$T_M(d) = T_M(\infty)\cdot[1 - 4\cdot\sigma_{cl}/(d\cdot\Delta H_M\cdot\rho_c)] \qquad (1)$$

with σ_{cl} being the surface tension between crystal and liquid phases, ΔH_M the heat of melting and ρ_c the density.

In Figure 3, the T_M values determined from the DSC scans seen in Figure 2a (black, solid squares) and 2b (red, solid circles) were plotted as a function of 1/d. For both sets of melting peaks, T_M shifts linearly with 1/d, as predicted by eq 1, and linear extrapolations to infinite d revealed $T_M(\infty)$. In the case of the set of data points obtained from the DSC scans seen in Figure 2a, the obtained $T_M(\infty)$ value is in perfect agreement with $T_{M,I}(\infty)$ of form I. The linear extrapolation of the data points obtained from the DSC scans seen in Figure 2b, however, yields a $T_M(\infty)$ value of about 143 °C, which is significantly lower than the bulk melting temperature of form II ($T_{M,II}(\infty) \approx 156$ °C) and in accordance with T_M values reported for form III grown in acetaminophen/methylcellulose mixtures ($T_{M,III}(\infty) \approx 139$ °C).[17] We conclude that isothermal crystallization of acetaminophen confined to CPGs at T_c = 80 °C yields nearly exclusively form III. This interpretation is supported by the room-temperature wide-angle X-ray scattering (WAXS) pattern of acetaminophen isothermally crystallized at T_c = 80 °C confined to a CPG specimen with a d value of 43 nm (Figure 4) that essentially corresponds to the WAXS pattern previously reported for form

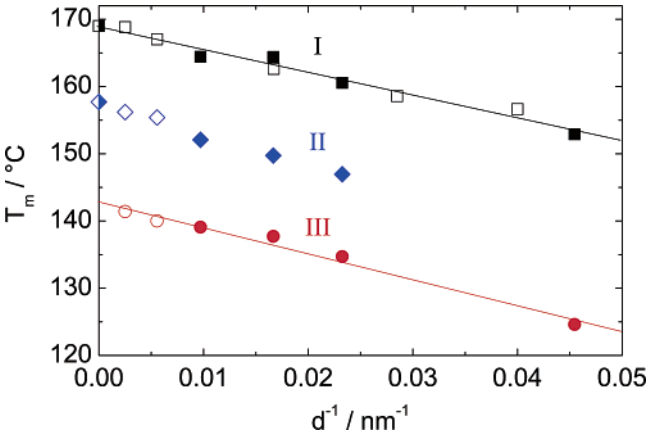

Figure 3. Gibbs−Thomson plots for acetaminophen confined to CPGs (solid symbols) and self-ordered porous alumina (open symbols). The melting point depression of different crystalline forms (I: squares; II: diamonds; III: circles) is shown. The T_M values correspond to the maximum of the melting peaks in the corresponding DSC heating scans. Bulk melting temperatures (half filled symbols) of form I and II are given for comparison. The lines are linear fits to the experimental data ($T_{M,I} \approx 169$ °C, slope $s_I = 340$ K·nm; $T_{M,III} \approx 143$ °C, slope $s_{III} = 386$ K·nm).

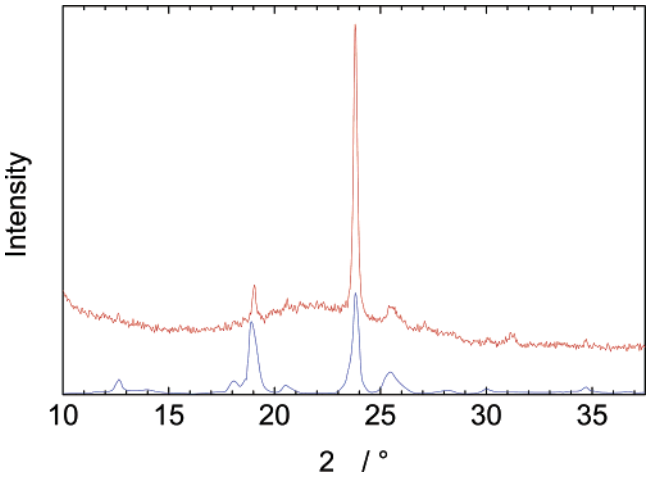

Figure 4. Room-temperature wide-angle X-ray scattering (WAXS) pattern for acetaminophen confined to a CPG specimen with d = 43 nm isothermally crystallized at 80 °C for 2 h (top). An amorphous halo belonging to the CPG matrix is clearly visible. The WAXS pattern for form III reported in ref 11 is shown for comparison at the bottom.

III crystals grown from solution inside glass capillaries.[11] We also determined the melting temperature $T_{M,II}$ of form II crystals confined in CPGs with d values of 43, 60, and 103 nm (Figure 3, blue solid diamonds). To this end, acetaminophen inside CPG specimens with d values of 43 and 60 nm was isothermally crystallized at $T_c > 100$ °C, where significant amounts of form II crystals form inside the nanopores. For $d = 103$ nm, $T_{M,II}$ could be determined from the DSC scan seen in Figure 2b. As expected, $T_{M,II}$ shifts linearly with $1/d$, and for specific d values, $T_{M,II}$ lies between the melting temperatures of forms I and III. Linear extrapolation of the data points belonging to form II reveals exactly the bulk melting temperature of form II ($T_{M,II}(\infty) \approx 156$ °C).

The T_M values obtained from additional DSC experiments on acetaminophen confined to self-ordered porous alumina membranes[18,19] with d values between 25 and 400 nm and pore depths of 100 μm are in line with those obtained from the CPGs. We infiltrated acetaminophen at 180 °C into self-ordered porous alumina and cooled it to room temperature while connected to a bulk acetaminophen surface film. Under these conditions, form I crystals rapidly grow even if the specimens are quenched in liquid nitrogen. DSC heating scans on the samples thus prepared after removal of bulk acetaminophen from the membrane surfaces show melting of form I at $T_{M,I}(d)$ values, which are in agreement with those observed for acetaminophen in CPGs (Figure 3, black open squares). Second heating scans were performed after heating the samples to 180 °C for 3 min, subsequent quenching to −40 °C, and crystallization at different T_c values. DSC scans of acetaminophen inside self-ordered porous alumina membranes with d values ranging from 180 to 400 nm show the coexistence of forms II (Figure 3, blue open diamonds) and III (Figure 3, red open circles). Again, the obtained $T_M(d)$ values are in excellent agreement with those obtained from CPGs. This is an interesting finding because it has been observed in ref 3 that the melting point depression of organic compounds in CPGs and nanoporous polystyrene is significantly different. However, the contact angles of liquid acetaminophen on glass (≈20°) and alumina (≈27°) at 170 °C are similar, indicating similar surface interaction. Thus, there seems to be no contradiction to the conclusion drawn by Ha et al.[3] that surface effects are important for $T_m(d)$ and an understanding of the crystallization in nanopores in general. We have clear evidence that the surface interaction is influencing which crystalline form of acetaminophen melts in a nanoporous host system.

Next, we estimate $\Delta H_{M,III}$ of form III. With $\Delta H_{M,I} \approx 186.1$ J/g,[7,10] the mass m of acetaminophen in each CPG specimen can be calculated from the $\Delta h_{M,I}$ values obtained from the first heating scans in Figure 2a according to $m = \Delta h_{M,I}/\Delta H_{M,I}$, assuming a degree of crystallinity of 100%. Now we can determine $\Delta H_{M,III} = \Delta h_{M,III}/m$ using the experimental $\Delta h_{M,III}$ values from the heating scans obtained after isothermal crystallization at $T_c = 80$ °C (Figure 2b) to 165−188 J/g for different d values. Alternatively, $\Delta H_{M,III}$ can be estimated by combining the Gibbs−Thomson equations for forms I and III: $\Delta H_{M,III} = T_{M,III}(\infty)\cdot s_I \cdot \Delta H_{M,I}/(s_{III}\cdot T_{M,I}(\infty))$, where s_I and s_{III} are the slopes in the Gibbs−Thomson plots for forms I and III. Assuming $\sigma_I/\rho_I = \sigma_{III}/\rho_{III}$, we can estimate $\Delta H_{M,III}$ to 154 ± 15 J/g, which is in line with the $\Delta H_{M,III}$ values estimated from Δh_M.

The results discussed above can be summarized as follows: T_M is exclusively determined by d for the investigated host−guest systems. Nonisothermal crystallization in contact with a bulk reservoir yields nearly exclusively form I for all d values, and isothermal crystallization at $T_c < 100$ °C in pores with $d < 60$ nm in the absence of a bulk reservoir yields exclusively form III. For $d > 100$ nm as well for $T_c > 100$ °C, mixtures of forms II and III are observed. For example, the amount of form II crystals in CPGs with d = 103 nm increases systematically with increasing T_c, while the form III fraction decreases (Figure 5). This is obvious from DSC heating scans after isothermal crystallization for 2 h at $T_c = 50$, 80, and 100 °C. At $T_c = 50$ °C, only form

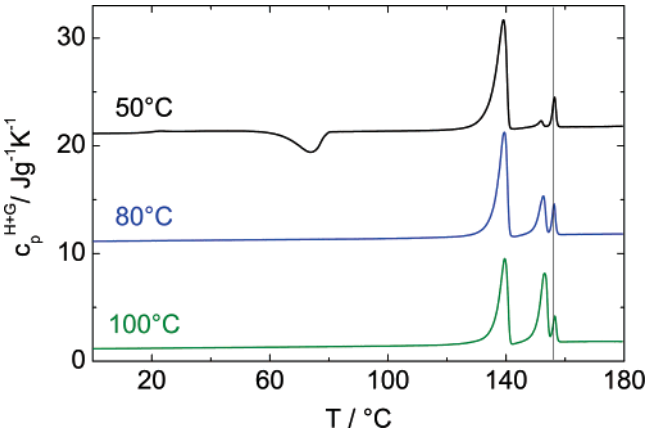

Figure 5. DSC heating scans (dT/dt = +10 K/min) of acetaminophen in CPGs (d = 103 nm) measured after isothermal crystallization at different temperatures T_c (50 °C: black; 80 °C: blue; 100 °C: green) for 2 h. The total heat capacity c_p^{H+G} of the host−guest systems is plotted. Melting peaks originating from form II crystals on the surface of the CPG specimens are denoted by the line at $T_{M,II} \approx 156$ °C. The curves are vertically shifted by 10 J/gK (T_c = 80 °C) and 20 J/gK (T_c = 50 °C).

III formed, while for T_c = 80 °C, a significant fraction of form II occurred. The portion of form II further increased at T_c = 100 °C. Therefore, the ratio of forms III and II might be controlled by T_c. The occurrence of form II for all d values larger than 100 nm may indicate the beginning of a transition to bulk behavior. In bulk systems, exclusively melting of form II is observed if acetaminophen is crystallized from the glassy state.[12]

Finally, we address the question why the metastable form III of acetaminophen is stabilized in nanoconfinement. Recently, Ha et al. proposed that this phenomenon is related to critical nucleus size.[2] These authors assumed that stable crystalline forms may have large critical nuclei that cannot be accommodated in nanoporous hosts. In contradiction to this idea, our results clearly show that not only the metastable form III but also the more stable forms II and I can grow inside the nanoconfinement depending on the conditions under which the crystallization takes place. This finding is in accordance with experimental results reported in refs 2,3, showing that different polymorphs of an organic compound can be formed in identical nanopores depending on the crystallization conditions. The observed stabilization of form III of acetaminophen in nanopores might have a kinetic or a thermodynamic origin. The solid−solid transition discussed for bulk acetaminophen in which form III converts to form II could be kinetically hindered. Arndt et al. have reported that the cooperative α-dynamics of confined liquids can significantly slow down in the proximity of pore walls with high surface energy.[20,21] Therefore, interfacial effects may affect the crystallization kinetics. Also, changes in equilibrium thermodynamics with respect to the corresponding bulk systems have to be taken into account.[22] Phenomena such as changes of the density of the confined acetaminophen or surface-induced effects in the vicinity of the pore walls may shift the conversion of form III into form II to higher temperatures. It was experimentally found that mesoscopic structure formation processes in nanoporous systems are often characterized by a complex interplay of competing effects. In the case of discotic liquid crystals confined to self-ordered porous alumina, the ordering of the mesophase in the vicinity of the pore walls is different from that in the center of the pores, and mesophase growth starting form the pore walls competes with mesophase growth guided by the geometry of the pores.[23] Also, the presence or absence of a bulk reservoir of the crystallizing species on the surface of the nanoporous host system influences the crystallization behavior.[24]

Yet, the reasons for the stabilization of metastable polymorphs in nanoconfinement are not understood. However, we could exploit this strategy to explore the thermodynamic properties of form III of acetaminophen, a polymorph which is unstable in the bulk. Crystallization of pharmaceuticals confined to nanoporous hosts systems will enable rational production and detailed characterization of metastable and transient crystal forms that could not be fabricated and studied otherwise. The use of nanoporous, biocompatible host systems is a simple, viable, and highly efficient strategy to stabilize and manipulate metastable polymorphs, potentially resulting in better controllable release characteristics of a next generation of drug delivery systems.

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