molecular pharmaceutics

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Surface-Enhanced Crystallization of Amorphous Nifedipine

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Received June 10, 2008; Revised Manuscript Received August 25, 2008; Accepted August 27, 2008

Abstract: Amorphous solids are generally more soluble and faster dissolving than their crystalline counterparts, a property useful for delivering poorly soluble drugs. Amorphous drugs must be stable against crystallization, for crystallization negates their advantages. Recent studies found that crystal growth in amorphous indomethacin is orders of magnitude faster at the free surface than through the bulk and this surface-enhanced crystallization can be inhibited by an ultrathin coating. Herein, we report a second system that exhibits the same phenomena. Crystal growth at the free surface of amorphous nifedipine (NIF) was at least 1 order of magnitude faster than that through the bulk below the glass transition temperature T_g (42 °C). A thin coating of gold (10 nm) reduced the surface crystal growth rate to the bulk crystal growth rate. Surface-enhanced crystal growth was more pronounced near and below T_g than substantially above T_g, which suggests that this growth mode is more important for the glassy state. Our results support the view that a thin layer of molecules near the surface have higher mobility than the bulk molecules and can enable faster crystal growth. The higher mobility of surface molecules and the resulting fast crystal growth can be suppressed by an ultrathin coating.

Keywords: Amorphous solid; glass transition; nifedipine; surface-enhanced crystal growth; coating

Introduction

Amorphous solids are generally more soluble and faster dissolving than their crystalline counterparts, a property useful for delivering poorly soluble drugs.[1] Developing amorphous drugs requires adequate understanding and control of their stability against crystallization because crystallization negates their advantages. Although the crystallization of amorphous drugs is often treated as a bulk problem and correlated with such bulk properties as viscosity and structural relaxation time, recent studies established the importance of surface-enhanced crystallization. The surface not only can serve as the preferred site for crystal nucleation[2] but also can enhance the rate of crystal growth.[3] Crystal growth at the free surface of amorphous indomethacin (IMC) is orders of magnitude faster than that through the bulk below the glass transition temperature T_g.[3] The surface-enhanced crystallization of amorphous IMC can be inhibited by an ultrathin coating of gold or polyelectrolyte.[4] These results identify a potentially important and presently neglected mechanism for the crystallization of amorphous drugs and a possible way to eliminate the mechanism.

Herein, we report a second example, amorphous nifedipine (NIF, Figure 1), that exhibits surface-enhanced crystallization. NIF is a calcium channel blocker and a model system

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10.1021/mp8000638 CCC: $40.75
Published on Web 09/23/2008

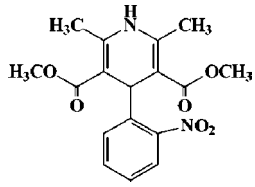

Figure 1. Molecular structure of nifedipine.

for studying the stability of amorphous drugs.[5−8] NIF (T_g = 42 °C) has approximately the same T_g as that of IMC but crystallizes faster than IMC, perhaps a result of its more symmetrical molecular structure (Figure 1). The fast crystallization of NIF makes it possible to compare the rates of crystal growth in both the surface mode and the bulk mode[8] over a wide range of temperature, which is difficult for IMC because of its slow bulk crystal growth. We observed that crystal growth at the free surface of NIF was at least 1 order of magnitude faster than that through the bulk. This surface-enhanced crystal growth could be suppressed to the bulk crystal growth rate by a thin coating of gold (10 nm). Surface-enhanced crystal growth was more pronounced near and below T_g than substantially above T_g, which suggests that this growth mode is more important for the glassy state. Our finding is relevant to understanding the type of molecular mobility responsible for the crystallization of amorphous NIF. In previous studies, the crystallization of amorphous NIF has been related to bulk molecular mobility exemplified by enthalpy relaxation time and NMR relaxation.[7] This study suggests that surface molecular mobility is another key factor for understanding the stability of amorphous NIF against crystallization.

Experimental Section

Nifedipine (1,4-dihydro-2,6-dimetyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate; NIF) was obtained from Sigma (St. Louis, MO) and used as received. To study surface-enhanced crystallization, 3−5 mg of NIF was melted on a clean 22 mm square cover glass at 185 °C for 2 min, covered with a 15 mm diameter round cover glass, and quenched to room temperature by contact with an aluminum block. The NIF layer thus formed was about 15 μm thick and confirmed to be free of crystals by polarized light microscopy. For some samples, the 22 mm square cover glass was detached from the NIF glass at room temperature by gently bending its center toward the round cover glass. This exposed a free surface of the NIF glass for studying surface-enhanced crystallization. For other samples, both the top and bottom cover glasses remained in place to study the bulk crystallization of amorphous NIF (confined between cover glasses).[8]

To measure the growth rate of crystals at the free surface, crystallization was initiated at 40 °C and allowed to continue at desired temperatures (4−80 °C). For temperatures between 45 and 80 °C, the sample was placed on a microscope hot stage (Linkam THMS 600) and measured in real-time. For temperatures between 4 and 40 °C, real-time observation was impractical and the sample was stored at a chosen temperature and periodically removed from storage for measurement at room temperature. Temperature control for this range was achieved with ovens (30 and 40 ± 1 °C), an air-conditioned laboratory (22 ± 1 °C), the reservoir of a circulating liquid cooler (10 ± 0.5 °C), and a walk-in cold room (4 ± 1 °C). The sample was protected from moisture by storage with Drierite.

A Denton Vacuum Desk II instrument (Denton Vacuum, Moorestown, NJ) was used to deposit a coating of 10 nm of gold on amorphous NIF. The deposition conditions were 50 mTorr pressure, 45 mA current, and 30 s deposition time. Under these conditions, the coating thickness was approximately 10 nm. Before coating, the liquid film of amorphous NIF looked yellow; after coating, the film looked green and was reflective. To study the nanocoating effect on existing surface crystals, we first initiated the crystal growth at the free surface at 40 °C and then coated the partially crystallized sample with 10 nm of gold. The samples were then stored at desired temperatures for growth measurement. Each reported growth rate is the average of several measurements with two or three independently prepared samples.

Polymorphs of NIF were identified with X-ray diffraction (Bruker D8 Advance) and Raman microscopy (Renishaw System 1000 equipped with a HeNe laser).[8]

Results

Of its three known polymorphs (α, β, and γ), only β NIF was observed in this study.[8] Figure 2 shows the Raman spectra of NIF crystals formed at the free surface and in the bulk (sandwiched between two cover glasses) of amorphous NIF; both spectra belong to β NIF. Figure 3 shows pictures of amorphous NIF crystallizing under three conditions: (1) at the free surface; (2) in the bulk; and (3) at the free surface covered with 10 nm of gold. Figure 3a shows a freshly made amorphous NIF sample between two microscope cover glasses, from which one cover glass (22 mm square) had been removed. The yellow, transparent liquid was now attached to only one cover glass (15 mm diameter round), exposing a free surface, and photographed against a dark

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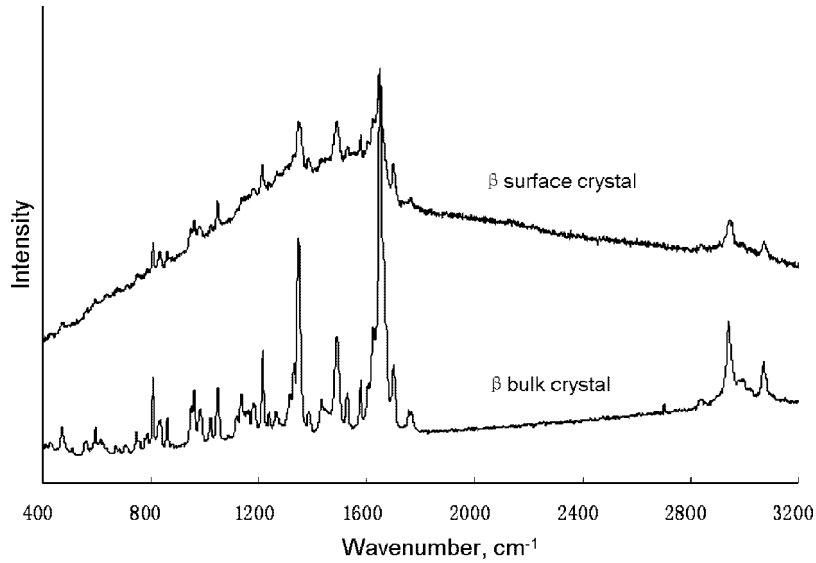

Figure 2. Raman spectra of NIF crystals grown in the bulk (confined between two cover glasses) and at the free surface. Both are the β polymorph.

background. Figure 3b shows the same sample as in Figure 3a after 1 day at 40 °C; by this time, the sample was fully covered by yellow crystals. Figure 3c shows a freshly made amorphous NIF sample between two cover glasses after 1 day at 40 °C; at this time, the sample was still largely amorphous, though early stage crystals were discernible (white spots). Figure 3d shows the same sample as in Figure 3c after 5 days at 40 °C. It is evident that crystal growth in the bulk (confined between two cover glasses) was substantially slower than that at the free surface (see later for quantitative data). Figure 3e shows a NIF sample with the following history: (1) it was freshly made between two cover glasses; (2) one cover glass was removed to expose a free surface; (3) the free surface was immediately coated with 10 nm of gold; and (4) the sample was stored for 1 day at 40 °C. Similar to the sample in Figure 3c, this sample was largely amorphous, but some early stage crystals were observable (white spots). These crystals grew to larger sizes (Figure 3f) after 5 days at 40 °C. It is clear that once the free surface was covered with 10 nm of gold, the crystal growth rate was approximately the same as that of the bulk sample (sandwiched between two cover glasses).

We confirmed the surface-enhanced crystallization of amorphous NIF by examining the cross section of a crystallized sample. For this experiment, we prepared a relatively thick NIF glass layer (about 1 mm) with a free surface, let it crystallize to full surface coverage at 30 °C, and cut the sample perpendicularly to the free surface. The cross section (Figure 4) shows birefringent crystals only next to the free surface, with the rest of the sample being amorphous. This result shows that the NIF crystals nucleated and grew at the free surface.

Figure 5 compares the typical morphologies of crystals grown at the free surface, in the bulk, and at the free surface covered with 10 nm of gold at 40 °C (below T_g) and 70 °C (above T_g). At temperatures below or near T_g, surface crystals displayed significantly different morphologies from those of bulk crystals (Figure 5a and b): Surface crystals were irregularly shaped and had a rough interface with the liquid; bulk crystals were spherulites and had a smooth interface with the liquid. At temperatures above T_g, surface crystals had morphologies similar to those of bulk crystals (Figure 5d and e). At all temperatures of this study, the morphology of crystals grown at a gold coated surface resembled that of bulk crystals.

Figure 6 shows crystal growth rates of amorphous NIF measured at the free surface, in the bulk, and at the free surface covered with 10 nm of gold. At 80 °C (T_g + 38 °C), the three rates were approximately the same. Below 70 °C, crystal growth at the free surface was faster than that through the bulk;[8] the difference was at least 1 order of magnitude below T_g. A coating of 10 nm of gold slowed crystal growth at the free surface substantially, to approximately the bulk level. The last result indicates that the growth at the free surface was indeed enhanced by the surface; otherwise, the thin gold coating should not have a strong inhibitory effect.

Discussion

Surface-Enhanced Crystallization of Amorphous NIF. This study shows that crystallization of amorphous NIF is substantially faster at the free surface than in the bulk, leading to a crystalline crust surrounding an amorphous interior (Figure 4). Crystal growth at the free surface is more than 1 order of magnitude faster than that in the bulk (Figure 6). This surface-enhanced crystal growth can be suppressed with a 10 nm coating of gold. NIF is the second system (to IMC[3,4]) for which surface-enhanced crystallization is observed.

NIF differs from IMC in that NIF crystallizes much faster. As a result, we were able to measure the crystal growth of amorphous NIF both at the surface and in the bulk below T_g and to observe the slowdown of surface crystal growth to the bulk level upon coating the surface with 10 nm of gold. (Below T_g, the bulk crystal growth of amorphous IMC is too slow to measure conveniently.[4])

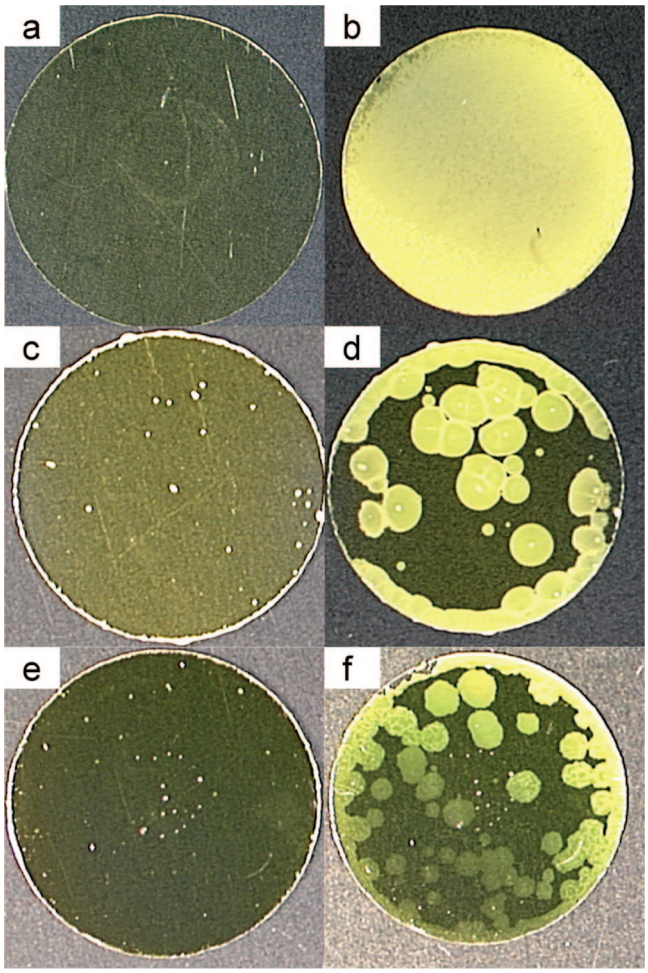

Figure 3. (a) Freshly made amorphous NIF sample between two microscope cover glasses, from which one cover glass had been removed. The sample now resides on one cover glass (15 mm round), exposing a free surface. (b) Same as (a) but after 1 day at 40 °C. (c) Freshly made amorphous NIF sample between two cover glasses after 1 day at 40 °C. White spots are early stage crystals. (d) Same as (c), after 5 days at 40 °C. (e) Freshly made amorphous NIF sample whose top surface was exposed and immediately coated with 10 nm of gold, after 1 day at 40 °C. Some white spots are early stage crystals; others are air bubbles. (f) Same as (e) after 5 days at 40 °C. The "shadow" was caused by a slight variation of sample thickness.

The inhibitory effect of an ultrathin coating on the growth of surface crystals[3,4] is consistent with the view that molecules at the free surface are more mobile than molecules in the bulk and "burying" the surface molecules under gold converts them to bulklike molecules. Other studies have demonstrated the existence of a thin mobile surface layer on an amorphous solid.[9−11] The slight difference between the growth rates observed in the bulk and at the free surface

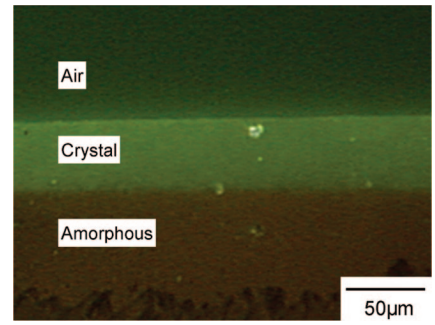

Figure 4. Cross-section of a thick NIF glass (about 1 mm) with a free surface. The sample had spent 6 days at 30 °C; after this time, the free surface was fully covered with crystals. The crystals were next to the free surface, surrounding an amorphous bulk.

covered with 10 nm of gold might arise from a slight but real difference between the two molecular environments in which crystal growth occurs.

An interesting feature revealed by Figure 6 is that the difference between surface and bulk crystal growth rates is important only near and below T_g; at higher temperatures, the two rates are indistinguishable. The relation between the two modes of crystal growth is also seen in crystal growth morphologies (Figure 5): at temperatures near and below T_g, the surface and bulk crystals have different morphologies; at higher temperatures, the surface and bulk crystals have similar morphologies. Based on data collected at $T_g + 60$ °C, Zanotto et al. concluded that the crystal growth rate of cordierite glass at the surface is the same as that in the bulk.[12] The results of this study suggest that the conclusion may not hold for temperatures near and below T_g, the region important for amorphous materials. It is possible that the difference between surface and bulk molecular mobility is more pronounced near and below T_g but diminishes at higher temperatures.[13] If this is true, surface-enhanced crystallization and bulk crystallization may be more readily distinguished near and below T_g but become difficult to distinguish at higher temperatures.

Relevance to Understanding Stability of Amorphous Drugs against Crystallization. The results of this study are relevant to understanding the relation of crystallization kinetics and liquid dynamics. Yoshioka et al. studied the relationship between the crystallization rates of amorphous nifedipine, phenobarbital, and flopropione and the molecular mobility in these matereials.[7] The fastest crystallization of

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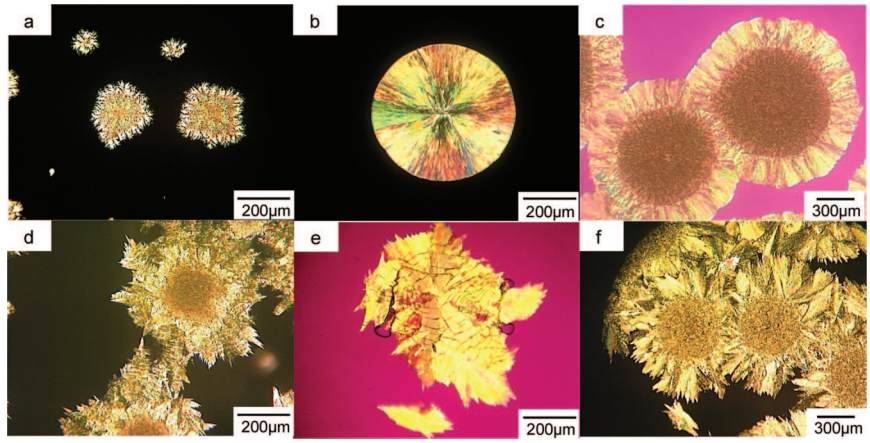

Figure 5. Morphologies of NIF crystals grown under different conditions. (a) At the free surface at 40 °C. (b) Through the bulk at 40 °C.[8] (c) At the free surface coated with 10 nm of gold at 40 °C. The dark central part was grown at 40 °C at the free surface; the sample was then coated with 10 nm of gold. Crystal growth continued under gold at a much slower rate to yield the brighter outer part, whose morphology is similar to that of the bulk crystal (b). (d) At the free surface at 70 °C. The central part crystallized at 40 °C, and the temperature was raised to observe further growth. (e) Through the bulk at 70 °C.[8] (f) At the free surface under 10 nm of gold at 70 °C. The central part crystallized at 40 °C.

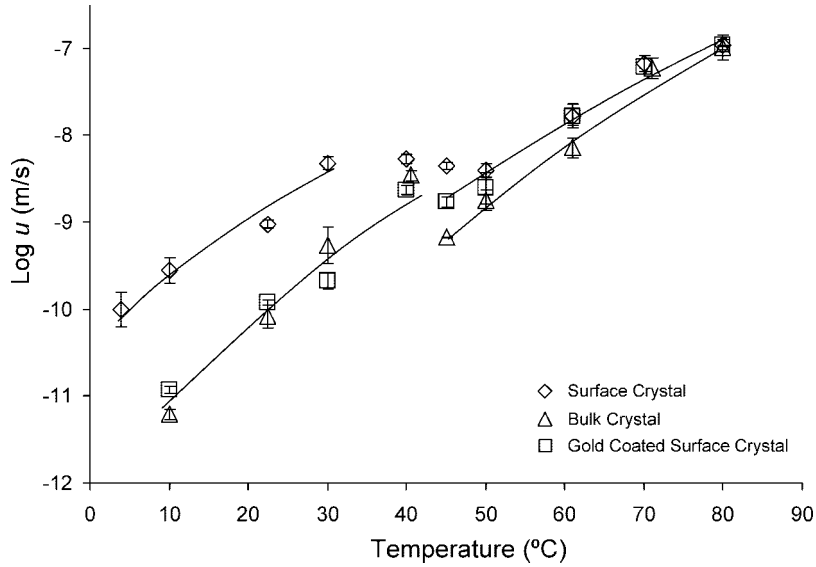

Figure 6. Growth rates of NIF crystals at the free surface, in the bulk (sandwiched between two cover glasses), and at the free surface under a 10 nm coating of gold (crystals first grown at the free surface at 40 °C and then coated with 10 nm of gold to observe further growth). Bulk growth rates above 20 °C have been reported;[8] the bulk growth rate at 10 °C is new data.

nifedipine is correlated with its highest molecular mobility as measured by enthalpy relaxation and ^{1}H NMR relaxation. The molecular mobility thus measured, however, is mainly the bulk molecular mobility, which may not be directly relevant for understanding surface-enhanced crystallization. Bhugra et al. have similarly attempted to correlate the crystallization kinetics of amorphous NIF with its bulk molecular mobility measured through enthalpy and dielectric relaxation.[14] It would be equally relevant to study the surface molecular mobility in order to understand the surface-enhanced crystallization, which controls the initial kinetics of crystallization when free surfaces are available.

The surface-enhanced crystal growth of amorphous NIF reported here is in reference to the bulk crystal growth that is already remarkably fast. At 40 °C ($\approx T_g$), the bulk crystal growth rate is 3×10^{-9} m/s,[8] which was measured with amorphous NIF sandwiched between two cover glasses (no free surfaces where crystals were grown). At this rate, ~400 layers of molecules are added to the crystalline phase in

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100 s, or one structural relaxation time of the liquid (the molecular diameter of NIF is assumed to be 8 Å). Such a high bulk growth rate makes NIF an example of organic materials that show fast crystal growth from glasses.[15−17] The activation of this fast mode of crystal growth near T_g causes the jump of growth rate in Figure 6 for data marked "Bulk Crystal" and "Gold Coated Surface Crystal".

Crystal growth in supercooled liquids is commonly assumed to be under diffusion control, for which the liquid can undergo substantial structural relaxation or diffusion during the time a molecular layer is added to the crystalline phase.[18] In contrast, for NIF and other systems exhibiting fast bulk crystal growth from glasses, the growth is so fast that no significant diffusion occurs in the bulk liquid during the time the crystal gains a new molecular layer. This so-called "diffusionless" crystal growth represents a substantial enhancement over the diffusion-controlled crystal growth observed at higher temperatures. Without involving significant diffusive motions in the bulk material, this mode of crystal growth probably occurs through the oscillatory motions available in glasses and viscous liquids.

In the case of NIF, the surface-enhanced crystal growth is yet another fast mode of growth, operative when free surfaces are available. For systems such as NIF, crystal growth rates predicted by the model of diffusion-controlled growth would be orders of magnitude too low because of the activation of two modes of crystal growth near T_g: diffusionless growth in the bulk and enhanced growth at the free surface. It is necessary to consider these two modes of crystal growth to understand and control the stability of amorphous drugs.

Conclusion

We identified a second system, amorphous nifedipine (NIF), that exhibits surface-enhanced crystallization. Crystal growth at the free surface of NIF was at least 1 order of magnitude faster than that through the bulk below the glass transition temperature T_g. A thin coating of gold (10 nm) suppressed the surface crystal growth rate to the bulk crystal growth rate. Surface-enhanced crystal growth was more pronounced near and below T_g than substantially above T_g, which suggests that this growth mode is more important in the glassy state. Our results support the view that a thin layer of surface molecules have higher mobility than the bulk molecules and can enable faster crystal growth. The higher mobility of surface molecules and the fast crystal growth they enable can be suppressed by an ultrathin coating. The finding is relevant to understanding and preventing the crystallization of amorphous drugs.

Acknowledgment. We thank the United States Department of Agriculture (2005-35503-16303) and AstraZeneca for supporting this work.

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