articles

molecular pharmaceutics

Crystallization of Amorphous Indomethacin during Dissolution: Effect of Processing and Annealing

Kristyn Greco and Robin Bogner*

Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, Connecticut 06269

Received January 26, 2010; Revised Manuscript Received May 3, 2010; Accepted May 11, 2010

Abstract: The crystallization of amorphous drugs during dissolution is a type of solution mediated phase transformation that can reduce the bioavailability enhancement one hoped to gain from the amorphous state. The goal of this study was to explore the effects of processing on the dissolution performance of amorphous indomethacin. The amorphous solids were prepared by four techniques, quench cooling the melted solid, cryogrinding γ indomethacin amorphous for 1 or 3 h and quench cooling the solid followed by 1 h of cryogrinding. Dissolution results assessed in a flow-through intrinsic dissolution apparatus reveal decreases in the dissolution rate of amorphous indomethacin during the experimental time frame indicating that a solution mediated phase transformation has occurred. The amorphous solids prepared by melt quenching and melt quenching followed by cryogrinding showed a significant dissolution rate advantage over the γ form of indomethacin. In contrast, indomethacin that was cryoground amorphous for 1 or 3 h did not show any dissolution rate advantage over the crystalline material. Transformation was confirmed by *in situ* Raman microscopy and polarized light microscopy with differences seen in the nature of the crystals apparent on the surface of the dissolving solid. A portion of the melt quenched amorphous sample was annealed at 25 °C and 0% relative humidity to induce partial crystallization of γ indomethacin. As crystallinity increased, the dissolution rate decreased. The transformation time of partially amorphous indomethacin was not dependent on the level of crystallinity present, indicating only a small fraction of crystalline material needs to be present to affect the kinetics of crystallization. The solution mediated phase transformation of amorphous indomethacin is affected by the processing method even though all solids were confirmed amorphous by polarized light microscopy and X-ray diffraction. Dissolution may distinguish differences in amorphous solids that other methods cannot discern.

Keywords: Solution mediated phase transformation; flow-through; Raman; phase conversion; glassy; ATR-FTIR

Introduction

The absorption of many potential therapeutic agents is hindered by low solubility and, consequently, low dissolution rate.[1] Dissolution is a particularly important determinant of the effectiveness of compounds that have high permeability, but low solubility.[2] With an increasing fraction of drug compounds proposed for development exhibiting poor water solubility and acceptable permeability (BCS class II compounds), there is a broader need to find approaches to improve solubility and bioavailability. Approaches to improve solubility and bioavailability include salt form selec-

* Corresponding author. Mailing address: University of Connecticut, 69 N. Eagleville Rd., Storrs, CT 06269. E-mail: robin.bogner@uconn.edu. Tel: 860-486-2136. Fax: 860-486-2076.

(1) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **2001**, *46* (1−3), 3–26.

(2) Lobenberg, R.; Amidon, G. L. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. *Eur. J. Pharm. Biopharm.* **2000**, *50* (1), 3–12.

10.1021/mp1000197
Published on Web 05/11/2010

tion, polymorph selection and forming amorphous solids. Solid form selection is used as a tool to increase the solubility of sparingly soluble solids which is expected to lead to an increase in bioavailability.[3,4] However, a more soluble solid form is not the most thermodynamically stable form. One drawback of using a metastable form is the propensity for reversion back to the stable, often less soluble form, particularly during dissolution. Therefore, the solubility advantage expected from an alternate form can be lost upon conversion by solution mediated phase transformation to a less soluble form during dissolution.[5−10] Solution mediated phase transformation causes a decrease in dissolution rate; this implies that solubility alone does not indicate whether a new drug form will improve oral absorption.[11]

Amorphous solids undergo solution mediated phase transformation to less soluble crystalline forms.[6,7] Therefore, formation of higher solubility amorphous solids may not increase the bioavailability of a drug in proportion with the solubility advantage.[11] Solution mediated phase transformations occur in three steps, (1) dissolution of metastable phase, (2) nucleation of stable phase, and (3) growth of stable phase.[12] This process can occur during dissolution even when bulk solution conditions are not supersaturated with respect to the stable form, because the concentration at the dissolving surface is equal to the solubility of the amorphous solid which is supersaturated with respect to the stable crystalline form under those conditions. Since the solution close to the surface is supersaturated, the stable crystalline form is likely to precipitate on the original solid covering the amorphous solid with a crystalline coating, thus eliminating the solubility advantage of the amorphous solid. These transformations have been well studied for polymorphic transitions.[6−8,10,12−19] The dramatically higher solubility advantage offered by amorphous forms leads us to study the solution mediated transformation of this important drug form.

Indomethacin was chosen as a model compound for the investigation of solution mediated phase transformation of amorphous compounds due to the wealth of information about the formation and characterization of the amorphous form.[4,6,20−30] Amorphous indomethacin can be prepared by milling under cryogenic temperatures and by quenching the melted solid in liquid nitrogen.[4,20] The solubility advantage of amorphous indomethacin has also been described for the

(3) Engel, G. L.; Farid, N. A.; Faul, M. M.; Richardson, L. A.; Winneroski, L. L. Salt form selection and characterization of LY333531 mesylate monohydrate. *Int. J. Pharm.* **2000**, *198* (2), 239–247.

(4) Hancock, B. C.; Parks, M. What is the true solubility advantage for amorphous pharmaceuticals? *Pharm. Res.* **2000**, *17* (4), 397–404.

(5) Chen Linna, R.; Wesley James, A.; Bhattachar, S.; Ruiz, B.; Bahash, K.; Babu Suresh, R. Dissolution behavior of a poorly water soluble compound in the presence of Tween 80. *Pharm. Res.* **2003**, *20* (5), 797–801.

(6) Debnath, S.; Predecki, P.; Suryanarayanan, R. Use of Glancing Angle X-Ray Powder Diffractometry to Depth-Profile Phase Transformations During Dissolution of Indomethacin and Theophylline Tablets. *Pharm. Res.* **2004**, *21* (1), 149–159.

(7) Murphy, D.; Rodriguez-Cintron, F.; Langevin, B.; Kelly, R. C.; Rodriguez-Hornedo, N. Solution-mediated phase transformation of anhydrous to dihydrate carbamazepine and the effect of lattice disorder. *Int. J. Pharm.* **2002**, *246* (1−2), 121–134.

(8) Rodriguez-Hornedo, N.; Lechuga-Ballesteros, D.; Wu, H. J. Phase transition and heterogeneous/epitaxial nucleation of hydrated and anhydrous theophylline crystals. *Int. J. Pharm.* **1992**, *85* (1−3), 149–162.

(9) Rodriguez-Hornedo, N.; Murphy, D. Significance of Controlling Crystallization Mechanisms and Kinetics in Pharmaceutical Systems. *J. Pharm. Sci.* **1999**, *88* (7), 651–660.

(10) Rodriguez-Hornedo, N.; Murphy, D. Surfactant-facilitated crystallization of dihydrate carbamazepine during dissolution of anhydrous polymorph. *J. Pharm. Sci.* **2004**, *93* (2), 449–460.

(11) Kaushal, A. M.; Gupta, P.; Bansal, A. K. Amorphous drug delivery systems: Molecular aspects, design, and performance. *Crit. Rev. Ther. Drug Carrier Syst.* **2004**, *21* (3), 133–193.

(12) Cardew, P. T.; Davey, R. J. The kinetics of solvent-mediated phase transformations. *Proc. R. Soc. London, Ser. A* **1985**, *398* (1815), 415–428.

(13) Aaltonen, J.; Heinanen, P.; Peltonen, L.; Korteiarvi, H.; Tanninen, V. P.; Christiansen, L.; Hirvonen, J.; Yliruusi, J.; Rantanen, J. In situ measurement of solvent-mediated phase transformations during dissolution testing. *J. Pharm. Sci.* **2006**, *95* (12), 2730–2737.

(14) Anquetil, P. A.; Brenan, C. J. H.; Marcolli, C.; Hunter, I. W. Laser Raman spectroscopic analysis of polymorphic forms in microliter fluid volumes. *J. Pharm. Sci.* **2003**, *92* (1), 149–160.

(15) Brittain, H. G. Fluorescence studies of the transformation of carbamazepine anhydrate form III to its dihydrate phase. *J. Pharm. Sci.* **2004**, *93* (2), 375–383.

(16) Caillet, A.; Puel, F.; Fevotte, G. In-line monitoring of partial and overall solid concentration during solvent-mediated phase transition using Raman spectroscopy. *Int. J. Pharm.* **2006**, *307* (2), 201–208.

(17) Tian, F.; Zeitler, J. A.; Strachan, C. J.; Saville, D. J.; Gordon, K. C.; Rades, T. Characterizing the conversion kinetics of carbamazepine polymorphs to the dihydrate in aqueous suspension using Raman spectroscopy. *J. Pharm. Biomed. Anal.* **2006**, *40* (2), 271–280.

(18) Wang, F.; Wachter, J. A.; Antosz, F. J.; Berglund, K. A. An Investigation of Solvent-Mediated Polymorphic Transformation of Progesterone Using in Situ Raman Spectroscopy. *Org. Process Res. Dev.* **2000**, *4* (5), 391–395.

(19) Zhang, G. G. Z.; Law, D.; Schmitt, E. A.; Qiu, Y. Phase transformation considerations during process development and manufacture of solid oral dosage forms. *Adv. Drug Delivery Rev.* **2004**, *56* (3), 371–390.

(20) Crowley, K. J.; Zografi, G. Cryogenic grinding of indomethacin polymorphs and solvates: assessment of amorphous phase formation and amorphous phase physical stability. *J. Pharm. Sci.* **2002**, *91* (2), 492–507.

(21) Fukuoka, E.; Makita, M.; Yamamura, S. Some physicochemical properties of glassy indomethacin. *Chem. Pharm. Bull.* **1986**, *34* (10), 4314–21.

(22) Taylor, L. S.; Zografi, G. Spectroscopic characterization of interactions between PVP and indomethacin in amorphous molecular dispersions. *Pharm. Res.* **1997**, *14* (12), 1691–1698.

(23) Taylor, L. S.; Zografi, G. The quantitative analysis of crystallinity using FT-Raman spectroscopy. *Pharm. Res.* **1998**, *15* (5), 755–761.

(24) Heinz, A.; Savolainen, M.; Rades, T.; Strachan, C. J. Quantifying ternary mixtures of different solid-state forms of indomethacin by Raman and near-infrared spectroscopy. *Eur. J. Pharm. Sci.* **2007**, *32* (3), 182–192.

neat solid theoretically and experimentally.[4,31] Such experiments result in a solubility advantage of approximately five times that of the most stable crystalline form. The solubility advantage is, however, short-lived due to the transformation to crystalline forms. It is our objective to understand the kinetics of the solution mediated phase transformation of indomethacin during dissolution in a flow through dissolution apparatus under controlled hydrodynamic conditions. The specific aims of this study were to evaluate the effect of processing (i.e., milling or melt quenching) and annealing on solution mediated phase transformation and to understand how conversion is affected by hydrodynamic conditions.

This study utilizes a flow cell for dissolution experiments (Figure 1) that was described previously.[32,33] The flow cell was designed to (1) utilize small sample size of 15 mg or less, (2) allow for real-time visual or microscopic inspection of changes in the sample, and (3) allow for a range of fully characterized hydrodynamic conditions at which the dissolution rate can be measured. The shear conditions of the flow-through apparatus are comparable to the shear rates for normal operating conditions of the USP type II[34,35] apparatus as well as *in vivo* estimates for shear rates as described by Abrahammson et al.[36,37]

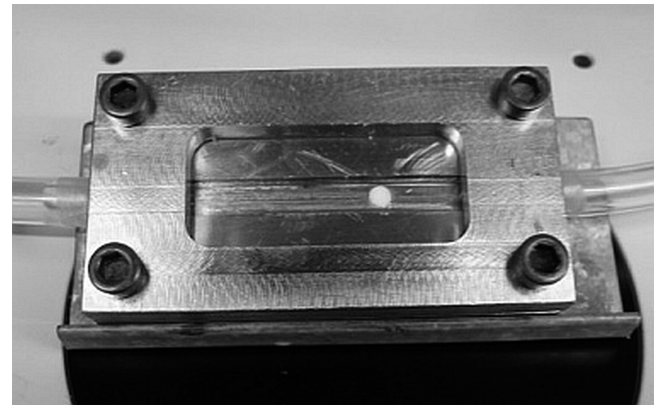

Figure 1. Picture of the dissolution flow cell.

Materials and Methods

Preparation of Amorphous Indomethacin Samples. Indomethacin (>99% TLC grade) was purchased from Sigma-Aldrich (St. Louis, MO) and found to be the γ polymorph. Amorphous and partially amorphous indomethacin samples were prepared by four methods. First, an amorphous form of indomethacin was prepared by melting the crystalline form at 165−168 °C and holding for 2 min; the melted drug was quenched cooled in liquid nitrogen. In a dry glovebag, the resulting glass was ground with a mortar and pestle and passed through a 150 μm screen. An alternate process for generating the amorphous form of indomethacin was by cryogenic milling (SPEX, Metuchen, NJ) of the crystalline form with an impact frequency of 10 Hz and 2 min of grinding followed by 2 min of cooling. Grinding was performed for a total of 1 or 3 h (2 or 6 h total time in cryogenic mill). In a third method, indomethacin was melt quenched as described above followed by cryogenic grinding for 1 h (2 h total); this process was performed to determine whether grinding has any effects on the melt quenched amorphous solid. An X-ray diffractometer (XDS 2000, Scintag Inc., Sunnyvale, CA) with Cu Kα radiation at 45 kV and 40 mA was used to evaluate the crystallinity of the sample at an interval of 0.02° and a scanning rate of 2°/min over a 2θ range of 5° to 40°. All of the above samples were found to be amorphous using this method and by polarized light microscopy. Lastly, melt quenched indomethacin was annealed at 25 °C and 0% relative humidity to induce partial crystallization of only the γ polymorph.[29] Subsequent crystallization was quantified using ATR-FTIR with data analysis using chemometric software as described below.

(25) Hedoux, A.; Paccou, L.; Guinet, Y.; Willart, J.-F.; Descamps, M. Using the low-frequency Raman spectroscopy to analyze the crystallization of amorphous indomethacin. *Eur. J. Pharm. Sci.* **2009**, *38* (2), 156–164.
(26) Savolainen, M.; Kogermann, K.; Heinz, A.; Aaltonen, J.; Peltonen, L.; Strachan, C.; Yliruusi, J. Better understanding of dissolution behaviour of amorphous drugs by in situ solid-state analysis using Raman spectroscopy. *Eur. J. Pharm. Biopharm.* **2009**, *71* (1), 71–79.
(27) Wu, T.; Sun, Y.; Li, N.; De Villiers, M. M.; Yu, L. Inhibiting Surface Crystallization of Amorphous Indomethacin by Nanocoating. *Langmuir* **2007**, *23* (9), 5148–5153.
(28) Wu, T.; Yu, L. Surface Crystallization of Indomethacin Below Glass Transition Temperature. *Pharm. Res.* **2006**, *23* (10), 2350–2355.
(29) Andronis, V.; Yoshioka, M.; Zografi, G. Effects of sorbed water on the crystallization of indomethacin from the amorphous state. *J. Pharm. Sci.* **1997**, *86* (3), 346–351.
(30) Bahl, D.; Bogner, R. H. Amorphization of Indomethacin by Co-Grinding with Neusilin US2: Amorphization Kinetics, Physical Stability and Mechanism. *Pharm. Res.* **2006**, *23* (10), 2317–2325.
(31) Murdande, S. B.; Pikal, M. J.; Shanker, R. M.; Bogner, R. H. Solubility advantage of amorphous pharmaceuticals: I. A thermodynamic analysis. *J. Pharm. Sci.* **2010**, *99* (3), 1254–1264.
(32) Bogner, R. H.; Bergman, T. L.; Greco, K.; Michaels, D. J.; Chawarski, S. J. Flow-through apparatus for microscopic investigation of dissolution of pharmaceutical solids. United States Patent Application 2008138261, 2008.
(33) Greco, K.; Bergman, T. L.; Bogner, R. H. Design and Characterization of a Laminar Flow-Through Dissolution Apparatus: Comparison of Hydrodynamic Conditions to Those of Common Dissolution Techniques. *Pharm. Dev. Technol.* **2010**, doi: 10.3109/10837450903499341.
(34) Baxter, J. L.; Kukura, J.; Muzzio, F. J. Hydrodynamics-induced variability in the USP apparatus II dissolution test. *Int. J. Pharm.* **2005**, *292* (1−2), 17–28.
(35) Kukura, J.; Baxter, J. L.; Muzzio, F. J. Shear distribution and variability in the USP Apparatus 2 under turbulent conditions. *Int. J. Pharm.* **2004**, *279* (1−2), 9–17.
(36) Abrahamsson, B.; Pal, A.; Sjoeberg, M.; Carlsson, M.; Laurell, E.; Brasseur, J. G. A Novel in Vitro and Numerical Analysis of Shear-Induced Drug Release from Extended-Release Tablets in the Fed Stomach. *Pharm. Res.* **2005**, *22* (8), 1215–1226.
(37) Pal, A.; Indireshkumar, K.; Schwizer, W.; Abrahamsson, B.; Fried, M.; Brasseur James, G. Gastric flow and mixing studied using computer simulation. *Proc. Biol. Sci.* **2004**, *271* (1557), 2587–94.

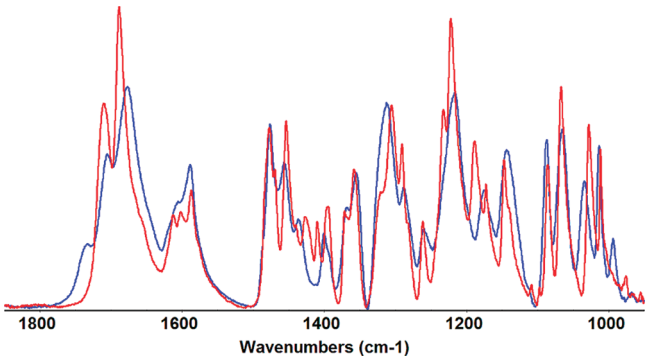

Figure 2. ATR-FTIR spectra of (red) γ polymorph of indomethacin and (blue) melt quenched amorphous indomethacin.

Differential Scanning Calorimetry. Solids were characterized by differential scanning calorimetry (DSC) immediately after preparation using TA Instruments (New Castle, DE) Q1000 with a scanning rate of 10 °C/min from 20 °C to 180 °C. Approximately 8 mg of solid was weighed into sealed hermetic pans.

Quantification of Crystallinity. Solid samples were analyzed for crystalline content using a Thermo Nicolet FTIR (Model Magna IR 560, Nicolet Instruments Technologies Inc., Westbury, WI) with ATR accessory equipped with diamond ZnSe crystal (Golden Gate, Specac, Cranston, RI) using OMNIC software. 64 scans were recorded with a resolution of 1 cm^{-1}. A chemometric technique (partial least-squares) described previously was utilized to generate the standard curve using TQ analyst software[30] (Thermo Scientific, Waltham, MA). Melt quenched amorphous indomethacin was mixed with proportions of γ crystalline indomethacin to generate a standard curve for analysis with ATR-FTIR to determine percent crystallinity by partial least-squares. Melt quenched samples were also confirmed to be amorphous by polarized light microscopy and powder X-ray diffraction. The FTIR spectra of amorphous and γ indomethacin show many distinct differences (Figure 2). The following spectral regions were used in the analysis, 1196−1183 cm^{-1}, 1430−1405 cm^{-1}, 1471−1441 cm^{-1} and 1713−1707 cm^{-1}. These regions correspond to ring bending and ring deformation of the chlorophenyl rings and the asymmetric stretch of the carboxylic acid dimer.[22] The chemical bonds described by these regions play a critical role in the hydrogen bonding essential to the crystal structure of indomethacin.[38] Melt quenched samples that were stored at 25 °C and 0% relative humidity were analyzed for crystalline content (γ polymorph) each week.

Dissolution. The dissolution apparatus is a channel flow apparatus consisting of an outer housing and insert assembled with a glass slide covering the insert (Figure 1). A previous report has described this flow cell using an insert with compact dimensions of 2 × 4 mm with a cross section of 4 × 2 mm.[32,33] The channel used in this study has a cross section of 1 × 6 mm (1 mm height and 6 mm width) and has a circular drug compact with a surface area of 12.6 mm^2. Although the dimensions of the insert used in the present study are slightly different than described previously,[33] the equations describing the hydrodynamics and mass transfer in the flow cell are the same. The mass transfer has been described by Shah et al.[39] The dissolution rate described by Shah et al. is expected to be constant throughout an experiment where the experimental parameters (solubility, diffusion coefficient, flow conditions) do not change with time, implying that the cumulative amount dissolved with time profile will be linear.[39] The fluid flow is simple flow in a rectangular duct and, at the flow rates used in this study, is laminar. For the flow rates utilized in this study, 0.5 mL/min and 7 mL/min, the Reynolds numbers (Re, which is a dimensionless number to describe laminar vs turbulent flow) are 2.4 and 34, respectively. These values for Re are much lower than the critical Re of 2300 (indicating transition to turbulent flow) for flow in a pipe or duct.[40] This is important since fluid flow in the GI tract has been suggested to be laminar.[36,37] In laminar flow, the layers of fluid flow over each other linearly and smoothly in adjacent layers, while in turbulent flow the layers interact with each other via turbulent eddies. This interaction between layers can cause temporary increases in local velocity which can add complexity to dissolution.[40] In order to avoid the turbulent flow regime, dissolution was performed with velocity well into the laminar flow regime. The concentration boundary layers have been calculated and found to be 250 μm for flow at 0.5 mL/min and 70 μm for 7 mL/min, which are within the dimensions of the apparatus.[41]

Indomethacin was compressed in the insert of the dissolution flow cell using a two ton press at 500 lb (ICL low ton press, Garfield, NJ) such that the indomethacin compact was flush with the channel surface. The surface area of the poorly soluble material remained constant throughout the experiment. Water was delivered to the flow cell via two syringe pumps with six 140 mL syringes (KDScientific syringe pump, Holliston, MA) at 0.5 and 7 mL/min. Therefore, two important variables for dissolution, surface area and hydrodynamics, were controlled such that dissolution rates of different solids could be directly compared. Currently there is no temperature control on the dissolution flow cell. Experiments were performed at room temperature (20−22 °C).

The concentration of indomethacin in the effluent from the dissolution flow cell was measured in fractions every 5

(38) Cox, P. J.; Manson, P. L. γ-Indomethacin at 120 K. *Acta Crystallogr., Sect. E: Struct. Rep. Online* **2003**, *E59* (7), o986−o988.

(39) Shah, A. C.; Nelson, K. G. Evaluation of a convective diffusion drug dissolution rate model. *J. Pharm. Sci.* **1975**, *64* (9), 1518–20.

(40) Geankoplis, C. J. *Transport Processes and Unit Operations*; Allyn and Bacon: 1978; 650 pp.

(41) Neervannan, S.; Reinert, J. D.; Stella, V. J.; Southard, M. Z. A numerical convective-diffusion of neutral compounds under laminar flow conditions. *Int. J. Pharm.* **1993**, *96* (1−3), 167–174.

or 10 min by HPLC (Thermo Finnigan HPLC, Waltham, MA). The mobile phase was 35% acetonitrile and 65% 0.1% trifluoroacetic acid in water. The flow rate was 1 mL/min. A Waters C18 Symmetry column was used with detection at 254 nm (Milford, MA). Retention time was 4 min, and the limit of quantification was 10 ng/mL.

Raman Microscopy. Raman spectra of the dissolving surface were collected during dissolution at 7 mL/min in the dissolution flow cell using a Thermo Nicolet Almega XR dispersive Raman equipped with a 780 nm laser at 100% power using a 20× objective (Thermo Scientific, Waltham, MA). The data were collected using a CCD detector with a resolution of 2 cm^{-1}. The laser spot size was 10 μm using the 20× objective.

Polarized Light Microscopy. Micrographs of the drug surface were taken during or immediately after dissolution using Olympus BX51 polarized light microscope with Hitachi camera (Olympus America Inc., Center Valley, PA). A 20× objective was used unless otherwise noted.

Results

Dissolution of X-ray Amorphous Samples. Dissolution of solids that undergo solution mediated phase transformation is often characterized by curvature in the cumulative amount dissolved vs time curves due to the change in dissolution rate during the experimental time scale.[42] The curvature is a result of precipitation of a more stable, less soluble form on the metastable form, e.g. the solubility of the surface exposed to the dissolution medium decreases with time, therefore decreasing the dissolution rate with time. This curvature is apparent in the dissolution of indomethacin melt quenched samples at 0.5 mL/min (Figure 3a). However, the curvature is less distinguishable in the samples that were cryoground for 1 h, which appear to follow a dissolution profile closer to the γ polymorph of indomethacin. Figure 3b shows the same data in terms of dissolution rate versus time, which is a clearer representation of how the dissolution rate changes with time. Melt quenched cryoground indomethacin has a dissolution rate advantage similar to that of the sample that was melt quenched, but not cryoground. There is a gradual decrease in the dissolution rates of both the melt quenched and melt quenched cryoground indomethacin which approach, but do not equal, the dissolution rates of the cryoground and crystalline samples. Although amorphous indomethacin that was prepared by cryogrinding for 1 and 3 h is indistinguishable from the melt quenched samples by PXRD and polarized light microscopy, the dissolution rate of the cryoground samples is markedly different from the melt quenched samples. Indomethacin that was cryoground for 1 h has no apparent dissolution rate advantage over the γ polymorph despite the fact that, by PXRD and polarized light microscopy, it is amorphous in nature. Indomethacin that was ground for 3 h has a slight dissolution rate advantage over the crystalline and 1 h cryoground samples. The advantage appeared to dissipate at 50 min where its dissolution rate approached the crystalline and 1 h cryoground samples.

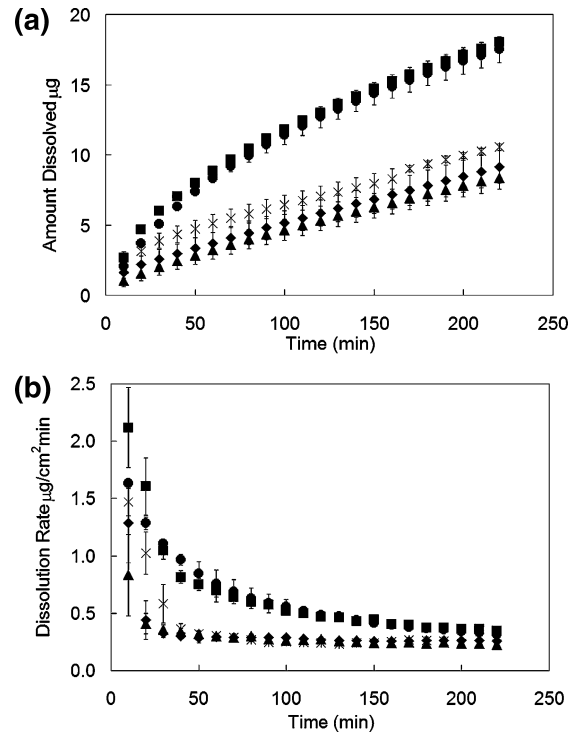

Figure 3. Dissolution of γ indomethacin and amorphous indomethacin prepared by melt quenching and cryogrinding at 0.5 mL/min in the dissolution flow cell: (■) melt quenched IMC, (●) melt quenched cryoground IMC, (×) 3 h cryoground IMC, (◆) 1 h cryoground IMC, and (▲) crystalline (γ polymorph) IMC. (a) Cumulative amount vs time. (b) Dissolution rate vs time.

Curvature in the cumulative amount profiles of melt quenched samples at the higher flow rate of 7 mL/min (Figure 4a) is comparable to that at 0.5 mL/min (Figure 3a). Again, the dissolution rates of melt quenched indomethacin and melt quenched cryoground indomethacin are higher than those of the cryoground amorphous samples and the γ crystalline form (Figure 4b). Indomethacin that was ground for 3 h had a slight dissolution rate advantage over the 1 h cryoground and γ polymorph. However, this advantage persisted only over the first 25 min of dissolution.

Dissolution rate profiles at both 0.5 mL/min and 7 mL/min (Figures 3b and 4b) show that dissolution rates decrease from the start of dissolution. For solids that undergo a solution mediated phase transformation, a decrease in the dissolution rate at $t = 0$ suggests that nucleation and growth of the stable crystalline form occurs on exposure to the dissolution medium. After a crystal form has nucleated, crystal growth inhibits the dissolution of the metastable, more soluble underlying amorphous form. However, the dissolution rate of crystalline indomethacin also decreases with time

(42) Curvature in the cumulative amount dissolved vs time curve has also been noted for dissolution of poorly soluble compounds that do not undergo solvent mediated phase transformation.[33] This curvature is not as pronounced as the curvature attributed to solvent mediated phase transformation and is hypothesized to be due to surface smoothing.

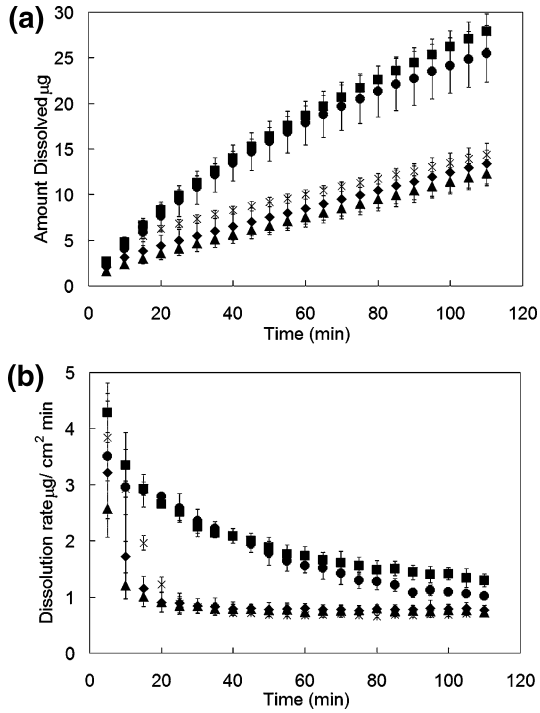

Figure 4. Dissolution of γ indomethacin and amorphous indomethacin prepared by melt quenching and cryogrinding at 7 mL/min in the dissolution flow cell: (■) melt quenched IMC, (●) melt quenched cryoground IMC, (×) 3 h cryoground IMC, (◆) 1 h cryoground IMC, and (▲) crystalline (γ polymorph) IMC. (a) Cumulative amount vs time. (b) Dissolution rate vs time.

even though no solid phase change is observed. This phenomenon has been described previously[43] and has been attributed to surface smoothing and disruption of fluid flow at the boundary surface due to surface roughness.[44] Other groups have circumvented this anomaly by using the same drug compact for successive dissolution experiments.[43] This procedure eliminates the decrease in dissolution rate for crystalline indomethacin (Figure 5). When studying solids that undergo solution mediated phase transformation, such as amorphous indomethacin, however, repeated use of a compact is not practical, since transformations appear to occur on a similar time scale to the decrease due to surface roughness. Therefore, in this study new compacts were generated for each experiment, including for γ indomethacin.

To analyze the time course of the dissolution profiles in a quantitative manner, an exponential equation, $R = A\ e(-t/\tau) + C$ was fit to the dissolution rate data (Table 1), where R is the dissolution rate, t is time, and τ is a characteristic time of conversion (describes when 63% of the conversion has taken place). The terminal dissolution rate corresponds to C, and the initial dissolution rate corresponds to $A + C$.

(43) Sun, W.; Larive, C. K.; Southard, M. Z. A mechanistic study of danazol dissolution in ionic surfactant solutions. *J. Pharm. Sci.* **2003**, *92* (2), 424–435.

(44) Grijseels, H.; De Blaey, C. J. Dissolution at porous interfaces. V. Pore effects in a parallel-plate dissolution cell. *Int. J. Pharm.* **1983**, *16* (3), 295–304.

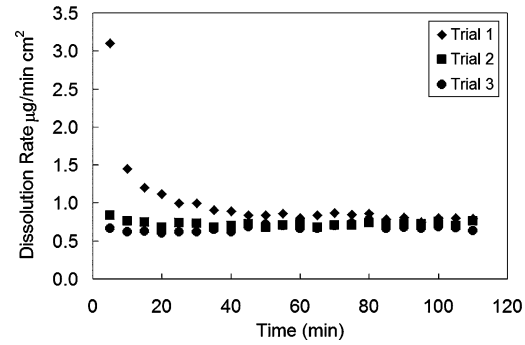

Figure 5. γ indomethacin dissolution in water, successive trials on the same compact, 7 mL/min.

Table 1. Parameters for Crystalline and Amorphous Indomethacin, Dissolution Rate, $R = A\ e(-t/\tau) + C$

	A (μg/min cm^2)	τ (min)	C (μg/min cm^2)
Flow Rate = 0.5 mL/min			
γ IMC	1.6 (1.5)[a]	11.7 (3.5)	0.26 (0.01)
MQ IMC	2.7 (1.0)	25.4 (8.6)	0.42 (0.03)
MQ cryoground IMC	1.5 (0.1)	48.8 (10.5)	0.33 (0.02)
cryoground 1 h (5.8% xtsal)	7.0 (2.5)	5.6 (1.9)	0.27 (0.02)
cryoground 3 h	2.4 (0.3)	16.0 (3.6)	0.24 (0.01)
Flow Rate = 7 mL/min			
γ IMC	6.6 (0.4)	3.9 (0.8)	0.78 (0.07)
MQ IMC	3.3 (0.3)	23.8 (5.0)	1.39 (0.14)
MQ cryoground IMC	3.1 (0.5)	49.5 (11.4)	0.70 (0.25)
cryoground 1 h (5.8% xtsal)	6.9 (1.4)	5.0 (1.4)	0.79 (0.08)
cryoground 3 h	5.6 (1.3)	9.8 (1.5)	0.66 (0.02)
Flow Rate = 0.5 mL/min			
MQ IMC 3.7% xstal	4.7 (1.4)	10.1 (2.6)	0.40 (0.06)
MQ IMC 24.5% xstal	2.9 (1.3)	9.7 (1.3)	0.29 (0.03)
Flow Rate = 7 mL/min			
MQ IMC 3.7% xstal	5.1 (1.1)	11.4 (2.2)	1.03 (0.09)
MQ IMC 24.5% xstal	6.2 (1.9)	9.3 (2.8)	0.94 (0.06)

[a] Standard deviation of $n = 3$.

This empirical equation quantifies how the dissolution rate changes with time characterizing the dissolution rates at time zero ($A + C$, before conversion occurs), terminal dissolution rates (C, final dissolution rate after most conversion occurs) and a time constant (τ) which give a measure of the extent and duration of a solubility advantage of a metastable solid which is of practical importance for oral absorption. The equation is based on observation of many dissolution curves and simply provides quantitative parameters for a complex process that is not fully understood. In examining the dissolution rate equation given by Shah et al.,[39] it is clear the dissolution rate changes due to changes in the solubility at the surface which occur due to crystallization in this case. A physical model of the decrease in dissolution rate will require additional knowledge of the decrease in solubility with time due to crystallization. This entails measurement of nucleation times, equilibrium solubility and growth rates of any polymorph that crystallizes to understand how fast the crystalline material covers the surface, and therefore affects dissolution. For practical purposes, the exponential equation provides parameters that can be used to compare

the conversion without measurement of physical quantities that may be difficult or time-consuming to evaluate (such as nucleation time and growth rates of each polymorph). The characteristic time of conversion, τ, associated with the γ polymorph of indomethacin during dissolution is representative of the time scale that surface smoothing takes place, not of a phase change (Table 1).

The τ value for dissolution of crystalline indomethacin at 0.5 mL/min is about three times the τ value at 7 mL/min indicating that the surface modifications that occur in the absence of a phase change are dependent on fluid velocity and mass transfer. In contrast to dissolution of crystalline indomethacin, melt quenched amorphous samples have similar τ values for conversion at 0.5 and 7 mL/min, indicating that a phase change is not dependent on fluid flow in the velocity range studied here. Additionally, τ values for melt quenched indomethacin at 0.5 mL/min and 7 mL/min are 2−6 times larger than for crystalline indomethacin, which allows easier differentiation of the phase conversion and surface smoothing processes. The dissolution of melt quenched cryoground indomethacin is characterized by τ values that are larger than that of melt quenched indomethacin, which suggests that crystallization progresses more slowly for this solid. Note that during compression of the melt quenched cryoground and melt quenched samples we observed a difference in the appearance in the compacted solids. The samples that had undergone cryogrinding appeared to be more translucent than the melt quenched samples. This difference may be due in part to a difference in porosity between the melt quenched and melt quenched cryoground samples. Such differences in porosity could lead to differences in dissolution behavior.

Characteristic times of conversion, τ, for indomethacin samples that were cryoground for 1 or 3 h to become amorphous are shorter as compared to melt quenched samples. The phase conversion occurs quickly in the case of cryoground materials as compared to melt quenched indomethacin, suggesting that the nature of the amorphous forms is different, which has been previously suggested for amorphous phases of griseofulvin and indomethacin.[20,45,46] For example, milled amorphous griseofulvin displayed different thermal behavior and X-ray diffraction patterns than melt quenched amorphous griseofulvin.[46] Indomethacin that was cryogenically milled to become amorphous displayed faster isothermal crystallization kinetics than melt quenched amorphous indomethacin.[20] The differences in melt quenched and cryoground indomethacin were also evaluated by XRD and analysis using a pair distribution function. The authors concluded that the material that was cryoground amorphous may have memory of the crystal structure, due to the method of processing.[47] Amorphous forms investigated in this study displayed differences in thermal behavior by DSC consistent with previous reports (Table 2).[20] The glass transition (T_g) temperatures for melt quenched amorphous and melt quenched cryoground amorphous indomethacin were slightly higher compared to the 1 and 3 h cryoground amorphous samples. Stresses during processing have been shown to produce shifts in T_g, which were found to be due to enthalpy recovery effects.[48] Using modulated DSC, however, the glass transition temperatures were not significantly different as previously reported.[48] The crystallization exotherms of the amorphous samples also differed depending on how the sample was prepared. The cryoground samples crystallized at lower temperatures than the melt quenched samples. The sample that was cryoground amorphous for 3 h displayed two exotherms, one at 81 °C and a broad exotherm at 110 °C. The cryoground and melt quenched cryoground samples show melting endotherms corresponding to the γ polymorph, while the melt quenched sample show melt endotherms corresponding to the α and γ forms. Although the samples were found to be amorphous by X-ray powder diffraction and polarized light microscopy, there are differences observed by DSC. The data in the present study are consistent with the hypothesis that not all X-ray amorphous states are the same. In this study, the differences are also characterized by different dissolution behavior.

Table 2. DSC Results for Amorphous Forms of Indomethacin

material	temp °C, glass transition	exotherms		temp °C, endotherms[b]
		temp °C	J/g	
1 h cryoground	39.7 (1.4)[a]	70.6 (1.9)	43.1 (0.2)	160.7 (0.3)
3 h cryoground	39.9 (0.6)	81.3 (1.3)	31.8 (4.2)	160.2 (0.3)
		110.1 (4.0)	13.6 (3.2)	
melt quenched cryoground	43.4 (0.5)	106.6 (0.7)	71.6 (0.6)	160.2 (0.3)
melt quenched	43.0 (0.4)	122.7 (1.1)	87.7 (6.3)	161.2 (0.3)
				154.5 (0.0)

[a] Standard deviation of $n = 3$. [b] Melting points for polymorphs of indomethacin have been reported to be 160.9 (0.8) °C for the γ form[48] and 151.4 (0.6) °C for the α form.[20]

In addition to differences seen by DSC, the sample that was cryoground for 1 h was found to have 2.3% crystalline content (γ polymorph) by ATR-FTIR, which is a more quantitative technique for indomethacin than PXRD or polarized light microscopy in this range.[30] The percentage of crystalline content found in the sample that was cryoground for 1 h is significant since the limit of detection of γ indomethacin by our ATR-FTIR method is approximately 1.2%. Furthermore after compression of the solid the crystalline content of the 1 h cryoground material was found

(45) Chamarthy, S. P.; Pinal, R. The nature of crystal disorder in milled pharmaceutical materials. *Colloids Surf., A* **2008**, *331* (1−2), 68–75.

(46) Feng, T.; Bates, S.; Carvajal, M. T. Toward understanding the evolution of griseofulvin crystal structure to a mesophase after cryogenic milling. *Int. J. Pharm.* **2009**, *367* (1−2), 16–19.

(47) Bates, S.; Zografi, G.; Engers, D.; Morris, K.; Crowley, K.; Newman, A. Analysis of Amorphous and Nanocrystalline Solids from Their X-Ray Diffraction Patterns. *Pharm. Res.* **2006**, *23* (10), 2333–2349.

(48) Bhugra, C.; Shmeis, R.; Pikal, M. J. Role of mechanical stress in crystallization and relaxation behavior of amorphous indomethacin. *J. Pharm. Sci.* **2008**, *97* (10), 4446–4458.

to be 5.8% (±0.6). This result was unusual given that amorphous indomethacin is not known to crystallize during compression.[49] Further investigation revealed that crystallization also occurred in the uncompressed powder during the time the sample was compressed and analyzed, indicating that the increase in crystallinity was due to exposure to ambient conditions during handling, not compression. Therefore it is estimated at the start of the dissolution experiment the crystalline content of the 1 h cryoground samples is 5.8% (±0.6). It has been established that the isothermal crystallization kinetics of cryoground indomethacin are much faster than melt quenched indomethacin. Our findings are consistent with that work.[20] The melt quenched, melt quenched cryoground and 3 h cryoground samples were found to be amorphous by ATR-FTIR after compression.

The 3 h cryoground indomethacin sample exhibits a larger τ value both at 0.5 mL/min and at 7 mL/min than does the 1 h cryoground indomethacin. These data indicate that the 3 h cryoground material takes longer to fully crystallize on the surface of the compact than the 1 h cryoground material. The solubility of the 1 h cryoground samples is expected to be lower than the 3 h cryoground sample due to the crystallinity measured at time zero (5.8% for the 1 h cryoground and 0% for the 3 h cryoground). Furthermore the crystallinity present at time zero in the 1 h cryoground sample may serve as sites for surface crystallization, reducing the overall time for conversion.

Raman microscopy was utilized to further investigate the apparent crystallization of amorphous indomethacin during dissolution. With the flow rate set at 7 mL/min, the laser was focused on the solid surface while dissolution was occurring. The Raman spectra of the crystalline and amorphous forms of indomethacin are distinct between 1525 and 1725 cm^{-1},[22,23,25] which make differences in the Raman spectra during dissolution relatively straightforward to interpret. The peaks in this region are where carbonyl vibrations are observed. The benzoyl carbonyl peak at 1696 cm^{-1} in the γ crystalline Raman spectrum is sharp, while this peak appears broader and shifted to 1680 cm^{-1} in the amorphous solid.[22,23,25]

The Raman spectra of dissolving samples of melt quenched indomethacin indicate that changes in the solid begin occurring between 10 and 20 min. However, crystallization is observed by light microscopy approximately 5 min before being detected by Raman spectroscopy (Figure 6), indicating that light microscopy is more sensitive to detecting crystallization than Raman spectroscopy for this sample. The delay in detection of the transformation from amorphous to crystalline indomethacin by Raman microscopy is most likely due to penetration of the laser into the amorphous bulk of the solid, which is semitranslucent. The detection of crystalline material on the surface of the compact by Raman microscopy is dependent on the thickness of that crystalline

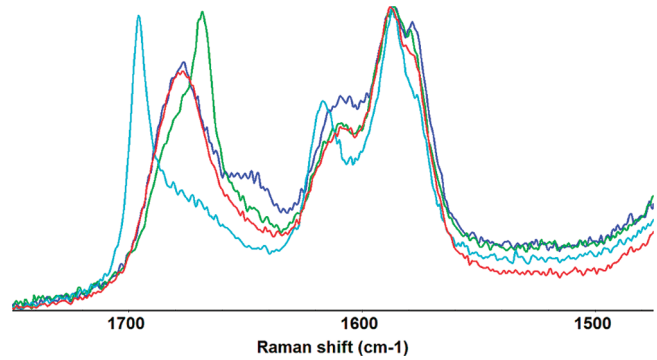

Figure 6. Raman spectra during dissolution at 7 mL/min in water of melt quenched indomethacin at (red) $t = 0$, (dark blue) $t = 20$ min, (green) $t = 45$ min, and (light blue) $t = 55$ min.

layer. When the crystalline layer is thin, the laser penetrates into the compact (to a depth that is material dependent and on the order of micrometers) and the crystalline signal is overwhelmed by that of the amorphous phase beneath it. Thicker crystalline layers are easier to detect by Raman microscopy. The microscope is operated in confocal mode, which improves detection of crystalline material at the surface of the dissolving solid. However, the crystalline layer of indomethacin that precipitates during dissolution is very thin in the initial stages of dissolution and cannot be detected even in confocal mode. This limitation suggests that the crystallization is occurring at the surface of compact rather than through the bulk of the compact. Surface crystallization would be expected since the solid is in contact with the dissolution medium at the immediate surface where two mechanisms for crystallization are feasible: (1) dissolution of the amorphous solid and recrystallization on the surface due to supersaturation with respect to the crystalline forms and (2) increased mobility (lower T_g) of the amorphous solid due to absorption of water. A compacted melt quenched sample was stored at 98% RH and qualitatively analyzed for crystalline material every 30 min (data not shown). Crystallization was apparent by polarized light microscopy at 90 min. During dissolution, we find crystals at the surface at 5 min, indicating that crystallization from a supersaturated solution may be a more significant mechanism during dissolution for this sample. Crystallization of similar compacted partially amorphous solids showed transformation through 10−20 μm of the compact,[6] however, Raman microscopy does not indicate this is the case for compacted samples studied in the present work.

During dissolution of melt quenched indomethacin the Raman spectra show development of a peak at 1650 cm^{-1}, corresponding to the α polymorph.[22,23] At 45 min the spectrum indicates a solid form present that is characterized by a sharp peak at 1670 cm^{-1}. This peak is not indicative of γ, α or amorphous indomethacin, nor does it correspond to the Raman spectrum of δ indomethacin.[25] At 55 min, the γ polymorph is observed at the surface characterized by the sharp peak at 1696 cm^{-1}. Thus, Raman spectra of dissolving melt quenched indomethacin confirm a solution mediated

(49) Okumura, T.; Ishida, M.; Takayama, K.; Otsuka, M. Polymorphic transformation of indomethacin under high pressures. *J. Pharm. Sci.* **2006**, *95* (3), 689–700.

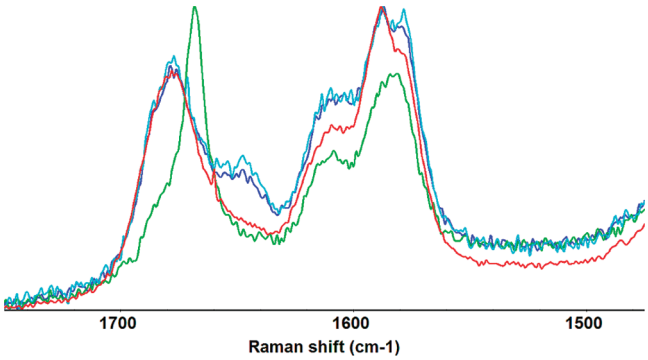

Figure 7. Raman spectra during dissolution at 7 mL/min in water of melt quenched cryoground indomethacin at (red) $t = 0$, (dark blue) $t = 20$ min, (green) $t = 40$ min, and (light blue) $t = 60$.

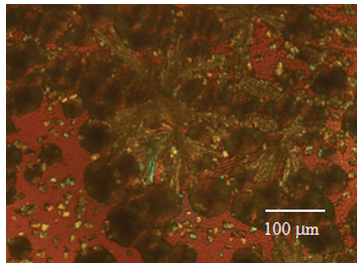

Figure 8. Polarized light microscopy after dissolution at 7 mL/min in water of melt quenched cryoground indomethacin.

phase transformation during dissolution to 3 distinct polymorphs of indomethacin.

The Raman spectra of melt quenched cryoground indomethacin (Figure 7) are similar to those of the sample that was simply melt quenched (Figure 6) and indicate that a solution mediated phase transformation begins to occur between 15 and 20 min from amorphous indomethacin to the α polymorph as evidenced by the shoulder at 1650 cm^{-1} (Figure 7). At 40 min, the appearance of the peak at 1670 cm^{-1} indicates that the unidentified form is present on the surface of the dissolving solid. Examination of the surface by polarized light microscopy after dissolution indicates three crystalline habits on the solid surface (Figure 8). The heterogeneous nature of the surface revealed by polarized light microscopy confirms that a Raman spectrum at one particular location on the compact surface is not representative of an average of the entire compact surface.[50,51] The laser spot size in which the Raman spectra are collected is

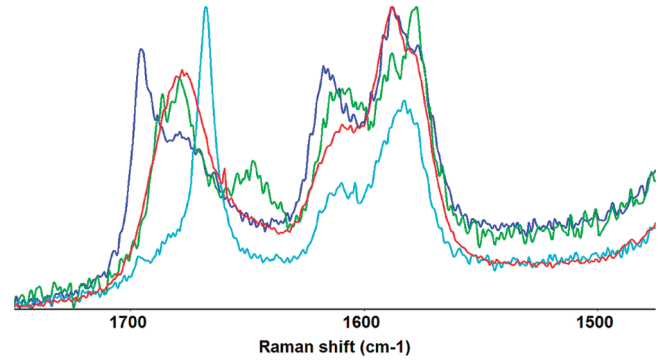

Figure 9. Raman spectra of melt quenched cryoground indomethacin surface after dissolution at 7 mL/min in water (red) amorphous $t = 0$, (dark blue) small rectangles, γ, (green) white hemispheres, α, and (light blue) needles, unidentified.

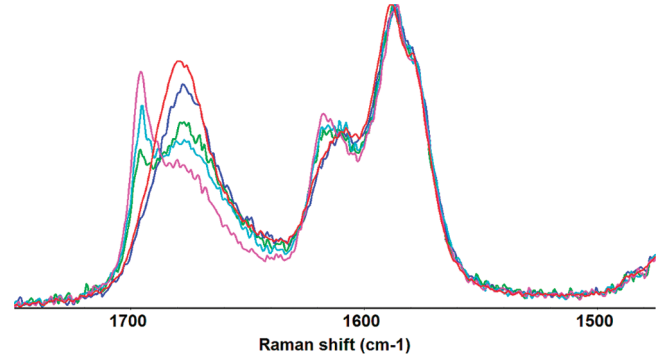

Figure 10. Raman spectra during dissolution at 7 mL/min in water of indomethacin cryoground for 1 h at (red) $t = 0$, (dark blue) $t = 5$ min, (green) $t = 15$ min, (light blue) $t = 30$ min, and (magenta) $t = 60$ min.

10 μm. Therefore, although the Raman data collected here are not indicative of the average Raman spectra of the entire surface (which would cover 12.6 mm^2), Raman microscopy is excellent for identification of polymorphs that have precipitated by focusing on the location of each crystal. The precipitated forms appear as hemispherical crystals, small rectangular crystals and needles. After dissolution, the crystal habits that were identified with polarized light microscopy were examined by Raman microscopy. The hemispherical crystals have Raman spectra corresponding to the α polymorph of indomethacin, the rectangular crystals correspond to the γ polymorph of indomethacin and the needles are the unidentified form that is characterized by the sharp peak at 1670 cm^{-1} (Figure 9). After the identification of the crystal forms by their morphology, we can more clearly see the reason that the γ form is difficult to detect *in situ*; due to the small crystal size the laser spot is "sampling" not just the γ crystalline form but the amorphous matrix on which it is growing.

The Raman spectra collected during dissolution of the samples that were cryoground for 1 h (Figure 10) exhibit dramatic differences in the crystallization compared to the melt quenched material. At $t = 0$, the Raman spectrum does not indicate that the γ polymorph is present in the compact, though by ATR-FTIR it is estimated to be 5.8%. At 15 min

(50) Langkilde, F. W.; Sjoeblom, J.; Tekenbergs-Hjelte, L.; Mrak, J. Quantitative FT-Raman analysis of two crystal forms of a pharmaceutical compound. *J. Pharm. Biomed. Anal.* **1997**, *15* (6), 687–696.

(51) The laser spot size for this instrument and the 20× objective is approximately 10 μm, which does not capture enough surface area to describe the entire compact surface. Others have remedied this problem for quantification of solid forms using Raman spectroscopy by using rotating stages.[50] This approach was not used here since quantification by Raman microscopy was not the intention for this study.

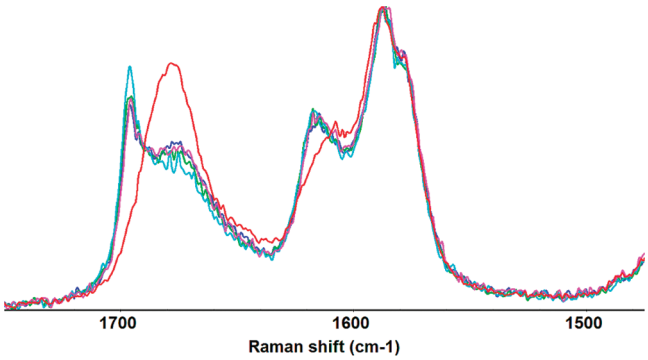

Figure 11. Raman spectra during dissolution at 7 mL/min in water of indomethacin cryoground for 3 h at (red) $t = 0$, (dark blue) $t = 5$ min, (green) $t = 25$ min, (light blue) $t = 45$ min, and (magenta) $t = 60$ min.

a shoulder at 1696 cm^{-1} becomes evident, indicating the appearance of the γ polymorph of indomethacin. As dissolution proceeds, the peak at 1696 cm^{-1} gradually increases with no indications of other polymorphs at the surface. After 75 min of exposure to water, the polarized light micrograph indicates only one crystalline form present on the dissolving surface, the γ polymorph (data not shown). The surface is remarkably homogeneous as compared with the melt quenched cryoground sample with γ crystals much more numerous, yet smaller in size. This visual observation suggests that more nuclei or crystallites are present at the surface of the cryoground material than the melt quenched material. Also, more surface of the cryoground amorphous solid appears to be covered with crystalline material at the end of the experiment than for the melt quenched material, which further hinders any solubility advantage of the amorphous form.

Raman data collected during dissolution of the sample cryoground for 3 h are similar to those of the 1 h cryoground material. The only form that crystallized during dissolution in water is the γ polymorph. The Raman data indicates the γ form first becomes apparent at 5 min (Figure 11). This result is consistent with the dissolution data which suggest that solution mediated phase transformation occurred within the first 10 min of the experiment. The layer of crystalline material on the surface of the cryoground samples appears to be very thin by microscopy and difficult to detect, thus Raman spectra were collected at the edge of the compact where the layer is thicker. Since the 3 h cryoground materials resulted in a semitransparent compact, it was possible to observe the sample using *in situ* polarized microscopy during dissolution (data not shown). Crystalline material was observed at the surface at 1 min into the experiment. At 20 min, the surface was completely covered by γ indomethacin, in agreement with dissolution data.

The differences in dissolution and crystallization of amorphous indomethacin prepared by melt quenching and cryogrinding were not predicted based on the PXRD, polarized light microscopy, and Raman spectroscopy which indicated all were indistinguishably amorphous solids prior to exposure to the dissolution medium. However, by DSC, differences were seen in the recrystallization behavior. ATR-FTIR indicated a small amount of crystalline material still present in the 1 h cryoground material immediately after cryogrinding that is not detected by any other method. Crystallization of the 1 h cryoground sample proceeds in ambient conditions during compression and assembly of the dissolution apparatus. The τ values and dissolution data indicate that the 3 h cryoground sample converts faster than the melt quenched samples, though all samples are amorphous. Dissolution appears to be very sensitive to process changes in preparing the amorphous solid.

Table 3. Quantification of Crystallinity of Amorphous Melt Quenched Indomethacin Stored at 25°C/0% RH

time (weeks)	% γ IMC
0	0
1	3.7 (0.7)[a]
3	24.5 (1.2)
6	67.0 (1.7)

[a] Standard deviation of $n = 3$.

Dissolution of Partially Crystalline Samples. Melt quenched amorphous indomethacin was annealed at 25 °C and 0% relative humidity. These conditions brought about crystallization of the γ polymorph of indomethacin as indicated by ATR-FTIR (Table 3). At a flow rate of 0.5 mL/min the dissolution profiles of partially crystalline samples show curvature in the amount vs time profiles, which is indicative of solution mediated phase transformation (Figure 12). As the percent crystallinity in the solid increases, the amount dissolved and dissolution rate decrease.

Melt quenched indomethacin allowed to crystallize to 3.7% has a dissolution rate advantage over the crystalline form that persists for more than 2 h (Figure 12). However, samples with 24.5% crystallinity show a remarkably reduced dissolution rate advantage. At 67.0%, any advantage is lost and the dissolution rate is similar to that of the crystalline form. At 7 mL/min, the dissolution rate advantage also decreases with an increase in crystalline fraction (Figure 13). However, in contrast to the data at 0.5 mL/min, the solid containing 24.5% γ polymorph does appear to maintain a dissolution rate advantage over the crystalline form. By plotting the ratio of dissolution rates of the partially amorphous and crystalline solids, we find no significant difference in dissolution rate advantage at the different flow rates due to high standard deviations in the first 10 min of each experiment (data not shown). The high standard deviation at early times of the experiments is likely due to sample compression; it was noted that the samples with crystalline fractions compressed differently (i.e., with more loose particle along the compact edges) than the melt quenched samples alone. The 1 h cryoground sample which is also partially crystalline (5.8%) was found to have a dissolution rate that was not significantly different from the γ crystalline material.

To quantitatively analyze the dissolution profiles of partially amorphous indomethacin (Figures 12 and 13), an exponential equation, $R = A\ e(-t/\tau) + C$ was fit to the

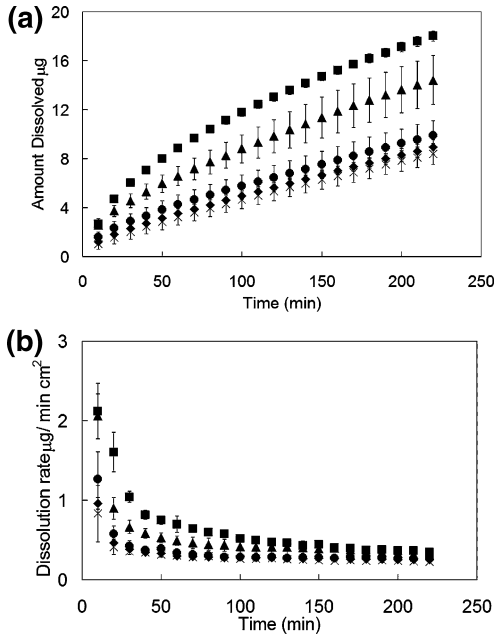

Figure 12. Dissolution of melt quenched amorphous indomethacin with 0%, 3.7%, 24.5%, 67.0%, and 100% crystallinity (t = 0, 1, 3, 6 weeks, γ crystalline) at 0.5 mL/min: (■) 0% crystallinity, (▲) 3.7% crystallinity, (●) 24.5% crystallinity, (◆) 67.0% crystallinity, and (×) 100% crystallinity. (a) Cumulative amount vs time. (b) Dissolution rate vs time.

dissolution rate data (Table 1). The characteristic time of conversion, τ, of the amorphous solid with 3.7% γ polymorph dissolving in water at 0.5 and 7 mL/min is less than half the τ for the melt quenched material. The conversion is much faster in the presence of even minute fractions of crystalline impurity (i.e., 3.7%). The characteristic time of conversion, τ, is not further reduced as crystalline content is increased to 24.5%, suggesting that only trace quantities of crystallinity are required for faster transformation. Above those trace quantities, the time course of conversion is independent of the crystalline fraction.

Raman microscopy during dissolution of partially amorphous indomethacin with 3.7% γ polymorph reveals two crystal forms present on the solid surface (Figure 14): the γ polymorph, characterized by the shoulder at 1696 cm^{-1}, and the unidentified form with a characteristic peak at 1670 cm^{-1}. At 10 min the peak at 1696 cm^{-1} appears as a small shoulder. This peak becomes more apparent at 60 min. The γ polymorph is certainly present at 3.7% at t = 0; however, the small amount of the γ form present is undetectable by Raman microscopy. It is clear that, as dissolution continues, the fraction of the γ form at the surface increases. At 45 min the unidentified form is present on the surface. This form was also found on the melt quenched amorphous surface during dissolution. In addition, the α polymorph of indomethacin was detected at the surface of the solid after the experiment was over (data not shown). The pattern of crystallization on the solid containing 3.7% γ polymorph is

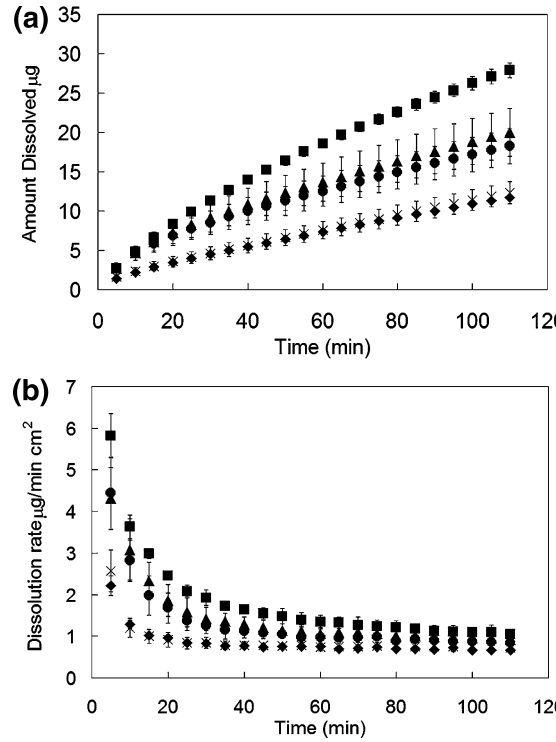

Figure 13. Dissolution of melt quenched amorphous indomethacin with 0%, 3.7%, 24.5%, 67.0%, and 100% crystallinity (t = 0, 1, 3, 6 weeks, γ crystalline) at 7 mL/min: (■) 0% crystallinity, (▲) 3.7% crystallinity, (●) 24.5% crystallinity, (◆) 67.0% crystallinity, and (×) 100% crystallinity. (a) Cumulative amount vs time. (b) Dissolution rate vs time.

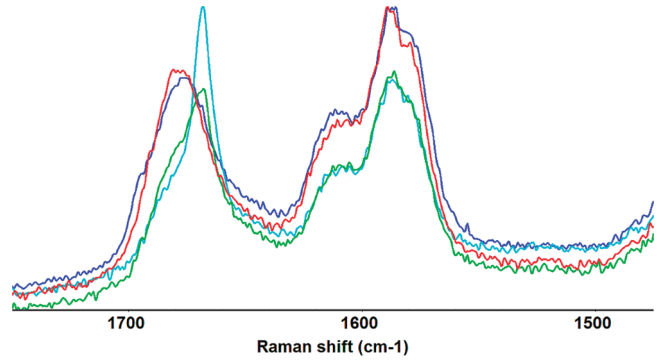

Figure 14. Raman spectra during dissolution at 7 mL/min in water of melt quenched indomethacin with 3.7% γ polymorph content at (red) t = 0, (dark blue) t = 10 min, (green) t = 45 min, and (light blue) t = 70 min.

similar to crystallization in the amorphous melt quenched sample, even though the time course of dissolution is different.

Figure 15 shows the Raman spectra during dissolution of partially amorphous indomethacin containing 24.5% γ polymorph. In this solid, the peak at 1696 cm^{-1}, characteristic of the γ polymorph of indomethacin, is evident on first exposure to the dissolution medium. As dissolution progresses, the peak at 1696 cm^{-1} increases, indicating the increase in the γ polymorph on the surface of the dissolving solid. After dissolution, the surface was examined by microscopy and

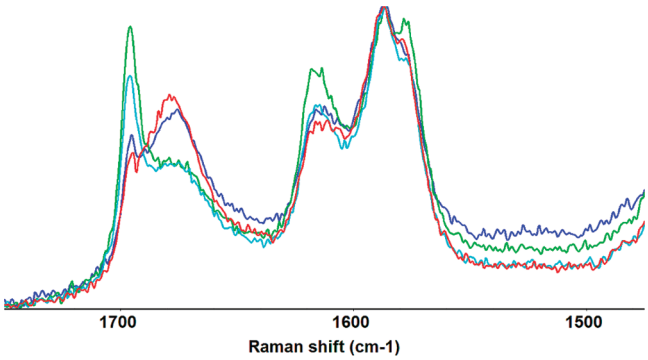

Figure 15. Raman spectra during dissolution at 7 mL/min in water of melt quenched indomethacin with 24.5% γ polymorph content at (red) $t = 0$, (dark blue) $t = 2$ min, (green) $t = 30$ min, and (light blue) $t = 60$ min.

the unidentified form with a characteristic peak at 1670 cm^{-1} was apparent at the surface of the compact. The α polymorph was not identified on the surface of the solid before or after dissolution. The presence of a large amount of γ polymorph may have hindered the growth of this polymorph. Raman spectra collected during dissolution of partially amorphous indomethacin with 67.0% γ polymorph indicate that only the γ polymorph of indomethacin is present on the surface during dissolution. This result also suggests that the presence of crystalline material in the partially amorphous samples hinders the crystallization of other polymorphs.

The flow rate over the solid was varied to assess the dependence of the solution mediated phase transformation on hydrodynamics. The values for τ at 0.5 and 7 mL/min for partially crystalline and amorphous solids prepared by different processing methods (Table 1) suggest that the solution mediated phase transformation of indomethacin is not dependent on the fluid flow during dissolution as the τ values are not significantly different at the two flow rates 0.5 and 7 mL/min. The solution mediated phase transformation of amorphous to crystalline indomethacin appears to be a surface phenomenon.

Summary and Conclusions

Amorphous indomethacin undergoes a solution mediated phase transformation during dissolution in water. This transformation was observed during dissolution in a flow through apparatus and confirmed by *in situ* Raman microscopy and polarized light microscopy. In all cases, differences in the time course of solution mediated phase transformation of indomethacin are more apparent from dissolution rate profiles, rather than cumulative amount dissolved. This analysis also allows the estimation of dissolution rates by fitting data by nonlinear regression to an exponential decay function. The estimation of dissolution parameters, A, C and τ, allows for quantifiable comparison of dissolution data.

Using this analysis, we find that solution mediated phase transformation of amorphous indomethacin is affected by the processing method. Melt quenched amorphous indomethacin (with or without cryogrinding) exhibits an initial dissolution rate advantage over the amorphous form that was prepared by cryogrinding. Although all materials were found to be amorphous by PXRD and polarized light microscopy with some differences seen by DSC, the dissolution profiles are very different. These results suggest that dissolution is very sensitive to changes in processing of amorphous solids. The characteristic times of conversion, τ, that were found by fitting the dissolution rate data to an exponential decay function reveal that the amorphous materials that were prepared by cryogenic grinding undergo solution mediated phase transformation faster than the melt quenched amorphous materials. The sample that was cryoground for 1 h was found to have residual crystallinity by ATR-FTIR that was not detected by other methods. The material crystallized with exposure to ambient conditions during handling, therefore, at the start of the dissolution experiment the crystalline content was estimated to be 5.8%. All other samples remained amorphous during the time scale of experimental preparation. The melt quenched materials were found to crystallize to the γ and α polymorphs of indomethacin as well as an unidentified form with a characteristic Raman peak at 1670 cm^{-1}. The materials that were cryoground amorphous were found to crystallize only to the γ polymorph. The difference in crystallization and dissolution of the melt quenched and 3 h cryoground samples (which are all amorphous) indicate that there are differences in the amorphous states that are not detected by PXRD or polarized light microscopy, but suggested by DSC.

Indomethacin annealed to various levels of crystallinity was also found to undergo solution mediated phase transformation during dissolution. The characteristic time of conversion was reduced in the presence of crystalline impurity. However this reduction in conversion time was not proportional to the fraction of γ indomethacin in the solid. Instead, above small fractions of crystallinity, the conversion times were indistinguishable. Additionally, the 1 h cryoground sample was also partially crystalline with 5.8% γ impurity at the start of the dissolution experiment. This material had the shortest conversion time (τ value) of the partially crystalline materials, indicating there are kinetic differences in solution mediated phase transformation based on processing of the amorphous solid even when crystalline impurity is present. The cryoground material was found to crystallize only to the γ polymorph while the material that was annealed to 3.7% and 24.5% crystallinity converted to more than one polymorph. Interestingly, as the γ crystalline fraction of the starting material was increased, the number of polymorphs that crystallized on the dissolving surface decreased. In the case of indomethacin, the variety of polymorphs that covered the amorphous or partially amorphous surface did not significantly affect the dissolution rate since all known polymorphs have similar solubilities.[4]

Solution mediated phase transformation of amorphous indomethacin was not affected by hydrodynamics in the range of conditions studied here. The characteristic times of conversion reveal similar kinetics of conversion of amorphous to crystalline indomethacin at 0.5 and 7 mL/min. This implies that the solution mediated phase transformation of

indomethacin is a surface phenomenon, i.e., convection or a concentration gradient does not result in a change in conversion time.

Acknowledgment. The authors would like to thank Sudhaker Garard and Riccardo Panicucci at Novartis Institutes for BioMedical Research (NIBR) for the use of the Raman and polarized light microscopes. The authors would also like to thank the Dane O. Kildsig Center for Pharmaceutical Processing Research for funding.

Supporting Information Available: Additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

MP1000197