

## Contact Line Crystallization To Obtain Metastable Polymorphs

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**ABSTRACT:** Crystallization of the metastable polymorph of paracetamol has been shown to occur around the edge of an evaporating aqueous solution. Conditions at the edge of a meniscus during evaporation led to an increase in solution concentration that allowed the nucleation of metastable crystals. This form was then unable to transform to the more stable polymorph via a solvent-mediated phase transformation because the crystals were removed from the solvent as the level of the solution dropped. These observations have been explained by analogies with previous reports of ring-shaped deposition, for example, salt crystallization and particle deposition from colloidal suspensions. This selective crystallization phenomenon is unlikely to be specific to paracetamol and may be applicable to the selective crystallization of metastable polymorphs of not only other pharmaceuticals but also other organic and inorganic materials. The conclusions drawn have particular relevance in the analysis of high-throughput crystallization experiments.

### Introduction

Crystallization is an important issue in the pharmaceutical industry. Drug properties such as solubility, dissolution rate, stability, hygroscopicity, and solid-state reactivity can all be affected by a change in the crystal structure or polymorph.<sup>1</sup> Therefore, the formation of a previously unknown polymorph during production or an uncontrolled transformation from one polymorph to another is highly undesirable. For this reason, in most commercial dosage forms, it is the thermodynamically stable polymorph that is used.<sup>2</sup> However, there is now increasing interest in using less stable, or metastable, polymorphs in systems with limited solubility or slow dissolution, to utilize improvements in these properties found with less thermodynamically stable crystal forms.

Several different methods have been tried to selectively crystallize a less stable polymorph of a drug. These include high-pressure crystallization,<sup>3</sup> spray drying,<sup>4</sup> and crystallization from the melt or a quenched amorphous state.<sup>5</sup> However all these methods are difficult to control precisely to avoid the formation of more than one polymorph and are generally limited to small volumes. The preferred industrial crystallization route is from solution, because the crystals tend to be of a higher purity and the process can be easily scaled up from the laboratory to much larger quantities.

However, although it is known that a less stable polymorph may nucleate first in solution due to a higher nucleation rate, it is energetically favorable for it to transform to a more stable polymorph over time.<sup>6–8</sup> Therefore, in many situations, it is difficult to isolate a metastable polymorph before it undergoes a solvent-mediated phase transformation to the more stable form.<sup>9,10</sup> Several studies have investigated the use of additives<sup>6,11</sup> and substrates<sup>12,13</sup> to either encourage one polymorph to nucleate preferentially or disrupt the growth processes of the other form. However, these methods have had only limited success in organic crystal systems because they do not take into account kinetic factors that are likely to play a dominant role during crystallization.

One potential route to obtain metastable polymorphs is by seeding<sup>14</sup> a saturated solution. However, there are a number of issues that must be considered. First, a suitable seed crystal must

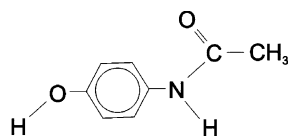
be obtainable by another method, for example, crystallization from the melt, and must be of sufficient purity. Second, once a solution has been seeded with a metastable polymorph, the newly formed crystals must still be harvested before a transformation occurs. Third, the crystals must be dried thoroughly, because any residual solvent will facilitate a transformation to a more stable polymorph at a later time.

Work in other fields has shown that crystallization can occur preferentially at the edge of an evaporating solution. Examples are the crystallization of salt around the edge of a drying drop<sup>15</sup> and crystalline arrangements of particles deposited from colloidal suspensions.<sup>16</sup> In this work, we investigated the effect of similar evaporation conditions on the crystallization of the polymorphic drug paracetamol (acetaminophen). The molecular structure is shown in Figure 1.

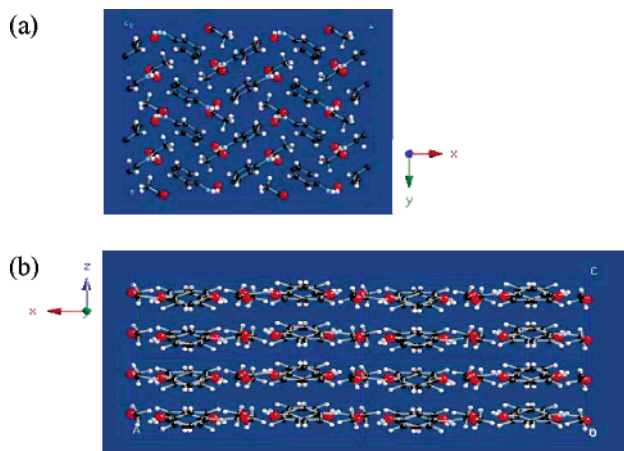
The crystal structures of two polymorphs of paracetamol are shown in Figure 2. Monoclinic form I is the more thermodynamically stable at ambient temperature. The two polymorphs are related enantiotropically, although the predicted transition temperature is well below 250 K.<sup>17</sup> Orthorhombic form II is metastable at ambient temperatures and can undergo a transformation to form I. However, it is the more stable polymorph at 0 K and is the denser of the two crystal structures. The greater stability of form I at room temperature is attributed to a greater entropy, not a lower energy packing arrangement.<sup>17</sup> The molecular layer arrangement of form II, with no interlayer hydrogen bonding, creates slip planes, which allow this polymorph to deform plastically. It has been shown that form II has better compaction properties than form I, in particular a lower yield pressure.<sup>10</sup>

Form I<sup>18</sup> is readily obtainable from solution and is used in commercial tablets. Metastable form II<sup>19</sup> has only been crystallized reproducibly from a cooled melt<sup>20,21</sup> or from saturated solutions seeded with form II crystals.<sup>22,23</sup> Form II has also been crystallized, less reproducibly, from solution by using specific additives or templating substrates. The effect of the addition of different polymers to an aqueous paracetamol solution was reported by Lang et al.<sup>24</sup> and Price et al.<sup>25</sup> Their results appeared to show that the ability to crystallize either form I or form II of paracetamol was dependent on the type of polymer that was present, and the stabilization of the metastable polymorph was attributed to the formation of specific interactions with the polymer substrates. However, the exact nature of these interac-

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**Figure 1.** Molecular structure of paracetamol.



**Figure 2.** Views of the crystal structures of (a) monoclinic form I and (b) orthorhombic form II paracetamol. Key to atoms: oxygen = red; carbon = black; hydrogen = white; nitrogen = pale blue.

tions was not specified. Mikhailenko et al.<sup>26</sup> successfully crystallized form II from solution without the use of additives; however the process was not reliable and the resultant crystals contained inclusions of water that facilitated a transformation to the more stable form I. In addition, no explanation was given for the formation of form II under the conditions imposed.

### Experimental Section

Acetaminophen (paracetamol) 98% was purchased from Sigma (U.K.). Distilled water was used in all experiments. Individual galvanized steel sample dishes were the cap section of a capped washer and were purchased from Baker & Finnmore (U.K.). The dishes were cleaned with a cotton bud soaked in ethanol before use.

**General Crystallization Method.** An amount of paracetamol (3–12.5 mg) was placed in a sample dish and 0.3 mL of water (at room temperature) was added. The dishes were placed in a custom-made aluminum insert in a block heater (UBD4, Grant Instruments Ltd., U.K.) preheated to a temperature between 40 and 80 °C. The solutions were heated for 4 min 40 s, and during this time, a known amount of water was added to replace that lost by evaporation. At the end of the heating time, the dishes were removed onto a cold metal block and cooled rapidly to room temperature. Solutions were left to freely evaporate at room temperature. Ten repeats were carried out for all experiments.

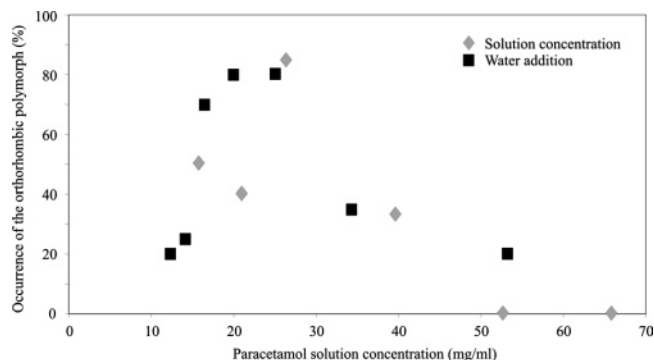
**Solution Concentrations.** Six initial solution concentrations were tested between 10 and 42 mg/mL. Solutions were heated to 80 °C with 0.1 mL of water added during heating.

**Water Addition.** An initial solution concentration of 16 mg/mL was used. Solutions were heated to 80 °C, and during heating, between 0 and 0.3 mL of water was added.

**Solution Heating.** An initial solution concentration of 16 mg/mL was used. Solutions were heated to between 40 and 80 °C. The amount of water added during heating corresponded to that lost by evaporation at that temperature.

**Solution Filtering.** This method was the same as that for solution heating, except the solution was passed through a 0.22 μm filter (Millipore 4 mm Millex syringe filter, nonsterile) before heating.

**Shaking.** An initial solution concentration of 16 mg/mL was used. Solutions were heated to 80 °C, and 0.1 mL of water was added during heating. After cooling on the cold metal block, samples were placed on a Rotamax 120, which produced a circular rotating motion throughout the evaporation process (shaking amplitude 20 mm at 100 rpm).



**Figure 3.** Occurrence of orthorhombic form II crystals depended on the postheating solution concentration. The required concentration was achieved in one of two ways. Either the initial (preheating) concentration was varied and the amount of water added during heating was kept constant (◆), or the initial solution concentration was kept constant and different amounts of water were added (■).

**Crystal Analysis. Raman.** The paracetamol crystals that formed were analyzed *in situ* using Raman spectroscopy. A Raman microscope was used with a ×50 long working distance lens. This produced a laser spot significantly less than 100 μm in diameter. An argon laser (wavelength 514.5 nm) was used. Spectra were collected over the extended range 50–3500 cm<sup>-1</sup> in a period of 10 s using Renishaw WIRE software. Paracetamol polymorphs were identified by peaks in the ranges 375–515 cm<sup>-1</sup> and 1210–1270 cm<sup>-1</sup>.<sup>27</sup>

**Photography.** Time-lapse photography was used to follow the crystallization process. For a number of samples, after cooling on the cold metal block, the dish was transferred to the stage of a microscope (Digital Blue QX5) connected to a computer. The magnification was set so that the whole dish was in the field of view and images were captured every 30 s for approximately 10 h. In addition, a number of still photographs were taken during crystallization using a digital camera.

### Results

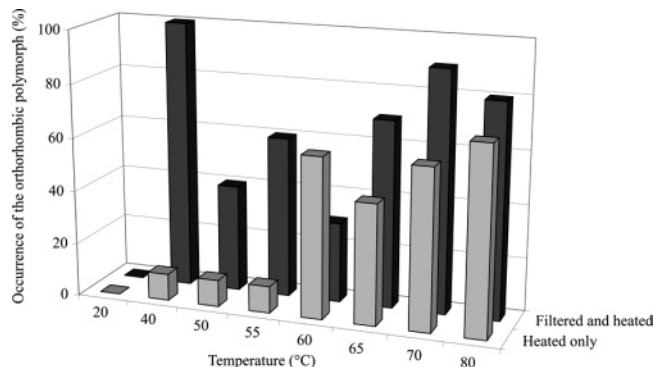
For the experiments in which the initial solution concentration or the volume of added water was varied, the results were interpreted by considering the postheating solution concentration. Measurements were made of the volume of water that was lost through evaporation during the heating step. Postheating solution concentrations were calculated using

$$\text{postheating solution concn} = \frac{\text{initial solution concn} \times 0.3}{0.3 - \text{vol water lost} + \text{vol water added}}$$

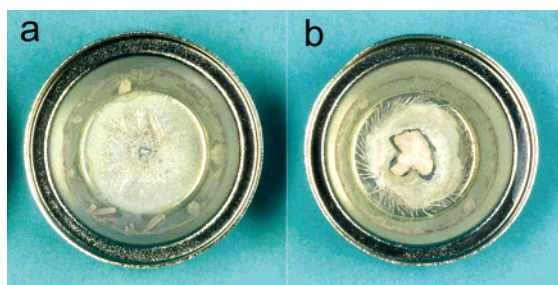
The results from these two experiments are shown in Figure 3. It was found that metastable form II crystals formed in the majority (more than 80%) of samples with a postheating concentration of between 20 and 26 mg/mL. Similar results were found using both methods. At concentrations below or above this range, the occurrence of form II dropped, and form I predominantly formed in dilute (<15 mg/mL) or very concentrated (>40 mg/mL) solutions.

When solutions with an initial concentration of 16 mg/mL were heated to temperatures lower than 80 °C, it was found that there was a decrease in the crystallization of form II (Figure 4). This occurred most dramatically below 60 °C, where form II crystallized in 60% of the samples heated to 60 °C, but in only 10% of the samples heated to 55 °C.

The heating step was present in the crystallization method to ensure that the initial form I paracetamol had fully dissolved and that the solution was brought fully into the undersaturated region of the solubility diagram.<sup>28</sup> It was likely that heating to



**Figure 4.** The growth of metastable orthorhombic form II depended on heating the solutions to above 60 °C if not filtered or to above 40 °C if larger form I seed crystals were first removed by filtering.



**Figure 5.** (a) Metastable form II paracetamol crystals formed around the sides of a sample dish, and (b) form I crystals formed on the base of a sample dish.

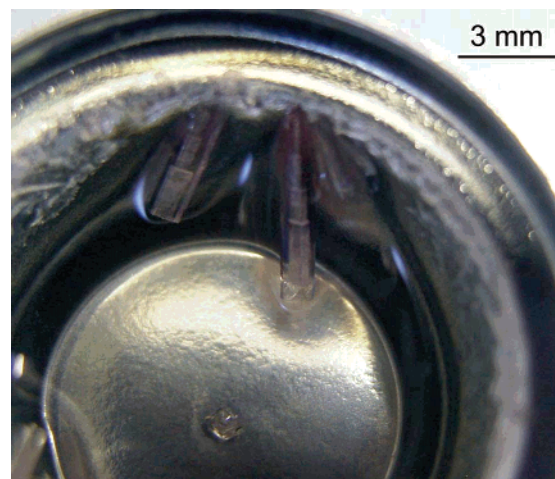
temperatures lower than 60 °C did not remove all of the form I seed crystals. These then acted as nuclei during the subsequent crystallization. This was supported by the results from experiments in which the solutions were filtered before being heated. The filter removed the larger seed crystals, and the solutions could then be heated to lower temperatures (Figure 4) to dissolve the remaining, smaller seed crystals and permit the growth of form II.

During all the experiments, it was noted that form II crystals always formed around the edges of the dishes, while form I crystals tended to form in the center (Figure 5). To understand where the form II crystals nucleated and how they grew in relation to the evaporating solvent, a series of experiments were conducted in which the crystallization process was followed by time-lapse photography. From these images, as well as stills taken at various times during crystallization (Figure 6), it was seen that the crystals nucleated around the edge of the meniscus, at the contact line, and grew out on the surface of the solution.

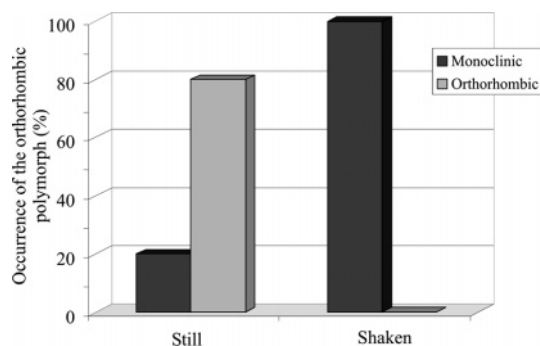
Results from samples in which the dishes were shaken constantly during evaporation, thereby disturbing the position of the contact line, are shown in Figure 7. Even though the solution concentration and heating regime were chosen to promote form II, only form I crystals grew in the dishes that were shaken.

### Discussion

An important step in the crystallization method was found to be the inclusion of a suitable heating step. This was required to produce an undersaturated paracetamol solution. When the solutions were subsequently cooled to room temperature, those solutions with concentrations greater than 26 mg/mL became highly supersaturated. Therefore, as soon as evaporation began, it was likely that either form I crystals formed rapidly or form



**Figure 6.** Photograph showing form II crystals that have nucleated at the contact line and are growing out on the surface of the solution.



**Figure 7.** The growth of orthorhombic form II was completely suppressed when the meniscus of the solution was disturbed during evaporation.

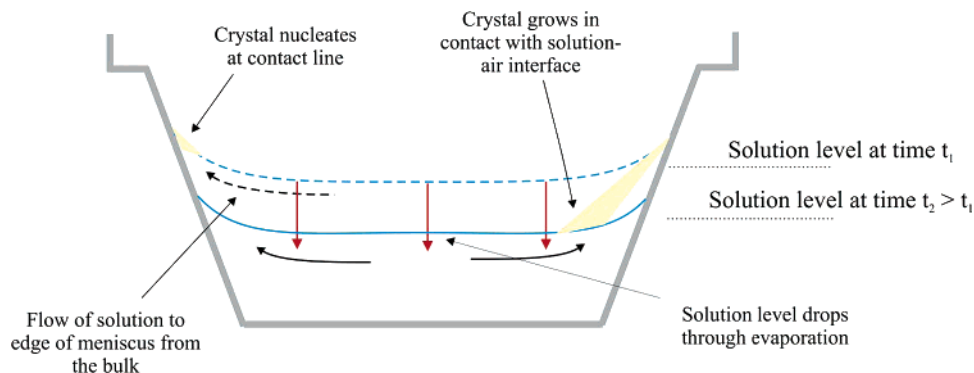
II crystals that nucleated transformed quickly to form I before crystallization was complete, that is, the solution had completely evaporated. At concentrations between 20 and 26 mg/mL, the solutions lie just beyond the solubility line. It is possible that at these concentrations, at room temperature, the solutions lie within the metastable zone, and therefore, crystallization of form II was more likely. At concentrations lower than 20 mg/mL, the solutions lie near the solubility curve but are still undersaturated at room temperature. However, as evaporation proceeded, the solution concentration increased and moved within the limits of the metastable zone. Form II was able to form in these samples, but not as reliably as with higher initial concentrations.

A proposed mechanism for the growth of form II is shown in Figure 8. The form II crystals nucleate at the edge of the meniscus and then grow in contact with the solution surface as the level of the solution falls through evaporation.

#### Why Did the Crystals Nucleate at the Contact Line?

Crystal nucleation at contact lines is already a well-known phenomenon, and examples include the crystallization of salt around the edges of puddles<sup>29</sup> and the formation of crystalline arrangements of colloidal particles by vertical deposition.<sup>16,30,31</sup> To understand these effects and explain the crystallization results in this work, it is necessary to have a good knowledge of the processes occurring at the edge of the meniscus.

The shape of a meniscus is determined by the fact that there is a driving force for liquids to minimize their surface area and when a liquid wets the walls of a container, that is, the contact angle is less than 90°, a concave meniscus is formed. With such



**Figure 8.** Schematic showing a proposed mechanism for the nucleation of metastable form II crystals and their growth at the edge of the meniscus.

a meniscus geometry, the evaporation rate increases toward the edge<sup>32</sup> and is greatest in the thin film region close to the container walls.

In this work, the saturated paracetamol solutions in the sample dishes formed concave menisci. The greater evaporation rate at the edges of the meniscus caused the solution to concentrate slightly in these regions. For concentrations between 20 and 26 mg/mL, the solution was already just beyond the solubility curve, and therefore, conditions were quickly reached in which nucleation occurred. Nucleation is also more energetically favorable in this meniscus region because there are two surfaces: the solution, and therefore the nucleating crystal, is constrained between the sides of the dish and the solution–air interface. Therefore, a smaller amount of the crystal surface is in contact with the solution, which may make nucleation more favorable.

However, in order for the crystal nucleus to have time to form, the edges of the solution must remain stationary for a finite length of time. This can occur due to contact angle hysteresis. Young's equation describes the equilibrium contact angle that forms on a flat surface,

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta$$

where  $\gamma$  is the interfacial energy, subscripts S, L, and V denote solid, liquid, and gas, respectively, and  $\theta$  is the contact angle.

In reality, surfaces are rough and are not chemically homogeneous, so the contact angle can vary from this value. The apparent angle that is measured when a liquid is spreading is known as the advancing contact angle,  $\theta_a$ , and the receding contact angle,  $\theta_r$ , is that that is measured as a liquid retracts. The difference between these angles is determined by the nature of the surface. An advancing or receding contact line will not move if  $\theta_r < \theta < \theta_a$ , and therefore, during this time, it can be said to be pinned. A further cause of contact angle hysteresis can be the presence of solutes in the liquid, which can change the nature of the solid–liquid interface and thereby affect the surface tension. For liquid evaporating from a drop or in a container with relatively rough surfaces, a certain amount of evaporation has to take place before the receding contact angle is achieved.<sup>33</sup> Then the contact line only has to move a relatively small distance before the roughness means that a larger contact angle is formed again. In this case, the contact line can move so slowly that it is effectively pinned in place. Pinning of the contact line has also been seen to occur on smooth surfaces.<sup>34</sup> In this case, deposition of particles occurred at the contact line due to the liquid layer being thinner than the particle diameter at this point, and only a few particles deposited on the substrate proved to be enough to pin the contact line.

In this work, because the solution concentration was such that nucleation was able to occur soon after the sample reached room temperature, it is possible that the meniscus edge did not move very far before a crystal nucleus pinned it in place. Once the crystal had formed at the contact line it was then only able to continue to grow as long as it was supplied with solute, in this case paracetamol molecules. A flow of liquid from the bulk of a solution to the edges of either a meniscus or a sessile drop has been utilized previously in several systems, for example, to form colloidal crystals, and is also the explanation given for the formation of ring-like stains when a drop of coffee dries.<sup>15,35</sup> Because water is lost faster from the edge of the meniscus and the contact line is effectively stationary, flows are set up from the bulk solution to the edges. Here it is hypothesized that the paracetamol molecules in solution were carried toward the edge of the meniscus by this flow, and this allowed the crystals to continue growing.

**Why Did the Form II Crystals Grow and Not Transform to Form I?** Although it is possible that the contact line remained stationary for a relatively short time but long enough for nucleation to occur, as evaporation proceeded the level of solution in the dishes dropped significantly.

At solution concentrations of 20–26 mg/mL, it is likely that conditions were such that form II crystallized first from solution and that this occurred at the contact line. Usually, the form II crystals would convert to the more stable form I *via* a solvent-mediated phase transformation; however, as the level of the solution dropped through evaporation, the water was effectively removed from around the crystals as they grew. This meant that they were then unable to transform to the more stable form I.

The form II crystals remained on the sides of the dish and could be removed in a dry state when evaporation was complete. It was found that these crystals remained in this form and did not revert to form I for several months.

## Conclusions

It has been shown that preferential crystallization of paracetamol at the contact line of an evaporating solution can lead to the growth of a metastable polymorph. Applying methods of contact line crystallization to polymorphic substances opens up the possibility of crystallizing metastable polymorphs reliably without the need for timely crystal retrieval before a transformation occurs or careful drying techniques. This phenomenon is unlikely to be one that is specific to paracetamol and, therefore, may be applicable to the selective crystallization of metastable polymorphs of other pharmaceuticals and also other organic and inorganic materials.

The findings in this work may also go some way to explain why other polymorphic forms are often found in high-throughput experiments.<sup>36–38</sup> In these methods, crystallization occurs in small volumes of solution contained within capillaries that are not usually agitated. It has previously been hypothesized that the small volumes and slower evaporation rates create high supersaturation levels where the precipitation of several crystalline forms becomes energetically favorable, while the smaller volumes decrease the likelihood of the presence of heterogeneous seed nucleants. However, in many cases, the vessel walls themselves can act as nucleation sites, as seen in this work, and attention should be given to where the crystals form in the sample wells. It is very easy to overlook those crystals that form around the edges of a dish.

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### References

- (1) Singhal, D.; Curatolo, W. *Adv. Drug Delivery Rev.* **2004**, *56*, 335–347.
- (2) Muller, M.; Meier, U.; Wieckhusen, D.; Beck, R.; Pfeffer-Hennig, S.; Schneeberger, R. *Cryst. Growth Des.* **2006**, *6*, 946–954.
- (3) Griesser, U. J.; Burger, A.; Mereiter, K. *J. Pharm. Sci.* **1997**, *86*, 352–358.
- (4) Beckmann, W.; Otto, W. H. *Chem. Eng. Res. Des.* **1996**, *74*, 750–757.
- (5) Stowell, G. W.; Behme, R. J.; Denton, S. M.; Pfeiffer, I.; Sancilio, F. D.; Whittall, L. B.; Whittle, R. R. *J. Pharm. Sci.* **2002**, *91*, 2481–2488.
- (6) Davey, R. J.; Blagden, N.; Potts, G. D.; Docherty, R. *J. Am. Chem. Soc.* **1997**, *119*, 1767–1772.
- (7) Schroer, J. W.; Ng, K. M. *Ind. Eng. Chem. Res.* **2003**, *42*, 2230–2244.
- (8) Zhang, G. G. Z.; Law, D.; Schmitt, E. A.; Qiu, Y. *Adv. Drug Delivery Rev.* **2004**, *2004*, 371–390.
- (9) Wang, F.; Wachter, J. A.; Antosz, F. J.; Berglund, K. A. *Org. Process Res. Dev.* **2000**, *4*, 391–395.
- (10) Nichols, G.; Frampton, C. S. *J. Pharm. Sci.* **1998**, *87*, 684–693.
- (11) Gu, C.-H.; Chatterjee, K.; Young, V., Jr.; Grant, D. J. W. *J. Cryst. Growth* **2002**, *235*, 471–481.
- (12) Mitchell, C. A.; Yu, L.; Ward, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 10830–10839.
- (13) Bonafede, S. J.; Ward, M. D. *J. Am. Chem. Soc.* **1995**, *117*, 7853–7861.
- (14) Beckmann, W. *Org. Process Res. Dev.* **2000**, *4*, 372–383.
- (15) Deegan, R. D.; Bakajin, O.; Dupont, T. F.; Huber, G.; Nagel, S. R.; Witten, T. A. *Nature* **1997**, *389*, 827–829.
- (16) Goldenberg, L. M.; Wagner, J.; Stumpe, J.; Paulke, B.-R.; Gornitz, E. *Langmuir* **2002**, *18*, 3319–3323.
- (17) Sacchetti, M. *J. Therm. Anal. Calorim.* **2001**, *63*, 345–350.
- (18) Haisa, M.; Kashino, S.; Kawai, R.; Maeda, H. *Acta Crystallogr.* **1976**, *B32*, 1283–1285.
- (19) Haisa, M.; Kashino, S.; Maeda, H. *Acta Crystallogr.* **1974**, *B30*, 2510–2512.
- (20) DiMartino, P.; Conflant, P.; Drache, M.; Huvenne, J. P.; Guyot-Hermann, A. M. *J. Therm. Anal.* **1997**, *48*, 447–458.
- (21) Politov, A. A.; Kostrovskii, V. G.; Boldyrev, V. V. *Russ. J. Phys. Chem.* **2001**, *75*, 1903–1911.
- (22) Al-Zoubi, N.; Malamataris, S. *Int. J. Pharm.* **2003**, *260*, 123–135.
- (23) Al-Zoubi, N.; Kachrimanis, K.; Malamataris, S. *Eur. J. Pharm. Sci.* **2002**, *17*, 13–21.
- (24) Lang, M.; Grzesiak, A. L.; Matzger, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 14834–14835.
- (25) Price, C. P.; Grzesiak, A. L.; Matzger, A. J. *J. Am. Chem. Soc.* **2005**, *127*, 5512–5517.
- (26) Mikhailenko, M. A. *J. Cryst. Growth* **2004**, *265*, 616–618.
- (27) Al-Zoubi, N.; Koundourellis, J. E.; Malamataris, S. *J. Pharm. Biomed. Anal.* **2002**, *29*, 459–467.
- (28) Granberg, R. A.; Rasmuson, Å. C. *J. Chem. Eng. Data* **1999**, *44*, 1391–1395.
- (29) Satterly, J. *Am. J. Phys.* **1956**, *24*, 529–530.
- (30) Cong, H.; Cao, W. *Langmuir* **2003**, *19*, 8177–8181.
- (31) Nozar, P.; Dionigi, C.; Migliori, A.; Calestani, G.; Cademartiri, L. *Synth. Met.* **2003**, *139*, 667–670.
- (32) Buffone, C.; Sefiane, K. *Int. J. Multiphase Flow* **2004**, *30*, 1071–1091.
- (33) Abkarian, M.; Nunes, J.; Stone, H. A. *J. Am. Chem. Soc.* **2004**, *126*, 5978–5979.
- (34) Deegan, R. D. *Phys. Rev. E* **2000**, *61*, 475–485.
- (35) Deegan, R. D.; Bakajin, O.; Dupont, T. F.; Huber, G.; Nagel, S. R.; Witten, T. A. *Phys. Rev. E* **2000**, *62*, 756–765.
- (36) Gardner, C. R.; Walsh, C. T.; Almarsson, O. *Nature* **2004**, *3*, 926–934.
- (37) Peterson, M. L.; Morissette, S. L.; McNulty, C.; Goldsweig, A.; Shaw, P.; LeQuesne, M.; Monagle, J.; Encina, N.; Marchionna, J.; Johnson, A.; Gonzalez-Zugasti, J.; Lemmo, A. V.; Ellis, S. J.; Cima, M. J.; Almarsson, O. *J. Am. Chem. Soc.* **2002**, *124*, 10958–10959.
- (38) Morissette, S. L.; Almarsson, O.; Peterson, M. L.; Remenar, J. F.; Read, M. J.; Lemmo, A. V.; Ellis, S. J.; Cima, M. J.; Gardner, C. R. *Adv. Drug Delivery Rev.* **2004**, *56*, 275–300.

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