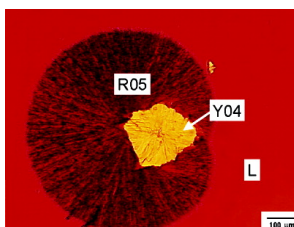


## Cross-Nucleation between ROY Polymorphs

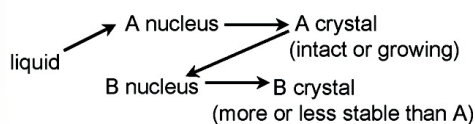
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**Table 1.** ROY Polymorphs

name	description	space group	mp, °C	year reported
Y	yellow prism	$P2_1/n$	109.8	1995 <sup>16</sup>
R	red prism	$P1$	106.2	1995 <sup>16</sup>
ON	orange needle	$P2_1/c$	114.8	1995 <sup>16</sup>
OP	orange plate	$P2_1/n$	112.7	2000 <sup>17</sup>
YN	yellow needle	$P1$	99	2000 <sup>17</sup>
ORP	orange-red plate	$Pbca$	97.4	2000 <sup>17</sup>
RPL	red plate	<i>a</i>	<i>b</i>	2001 <sup>18</sup>
Y04	yellow (2004)	<i>a</i>	<i>b</i>	2005 <sup>19</sup>
YT04	yellow prism	$P2_1/n$	106.9	2005 <sup>19</sup>
R05	red (2005)	<i>a</i>	<i>b</i>	this work

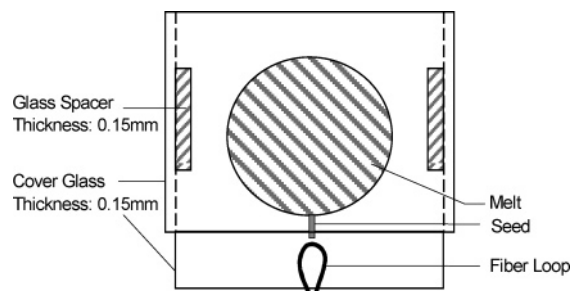
<sup>a</sup> Structure not yet solved. <sup>b</sup> Polymorphic conversion before melting.

nitrophenyl)amino]-3-thiophenecarbonitrile. This compound, dubbed ROY for its red, orange, and yellow crystals, has seven polymorphs of solved structures (Y, YN, YT04, ON, OP, ORP, and R) and two others (Y04 and RPL) whose structures have not been solved (Table 1).<sup>16–19</sup> ROY is currently the top system in the Cambridge Structural Database<sup>20</sup> for the number of polymorphs of solved structures. ROY is suitable for this study because it has many polymorphs of different colors, known structures, and known thermodynamic relations. We studied how cross-nucleation between polymorphs depends on the temperature, polymorph, relative stability and growth rates of polymorphs, and surface characteristics of seed crystals. This work focused on melt crystallization for the simplicity of a one-component system (no complications from solvent–solute interaction and solution-mediated polymorphic transformation). We observed extensive and sometimes selective cross-nucleation between ROY polymorphs. Certain polymorphs could not nucleate without the aid of others. The new polymorph was found to be more or less thermodynamically stable than the initial one but to always grow faster than or as fast as the initial one. The temperature and surface characteristics of the initial polymorph affected the occurrence of cross-nucleation. This study also identified a new metastable polymorph of ROY, the 10th of the family.

## Experimental Section

ROY (as polymorphs Y and R) was a gift from Eli Lilly and Co. Hot-stage microscopy was performed with a Linkam THMS 600 hot stage and a Nikon Optiphot2-Pol light microscope. Raman microscopy was performed with a Renishaw System 1000 micro-Raman spectrometer equipped with a HeNe laser and peltier-cooled CCD detector. With a 50× objective, it offered a spatial resolution of 0.5 μm. Powder X-ray diffraction was performed with a Bruker D8 Advance diffractometer (Cu Kα radiation, voltage 40 kV, and current 40 mA). Samples were placed on a zero-background silicon (510) sample holder and scanned from 2° to 50° (2θ) at a speed of 1.2° (2θ)/min and a step size of 0.02° (2θ). Differential scanning calorimetry (DSC) was conducted in crimped aluminum pans using a TA Instruments Q1000 unit under a 50 mL/min N<sub>2</sub> purge.

Seeding experiments were carried out using the setup illustrated in Figure 1. Typically, about 6–8 mg of ROY was melted on a cover glass (the bottom cover glass with preglued spacers) placed on a Linkam THMS 600 hot stage. Another cover glass was then placed on the melt,



**Figure 1.** Experimental setup employed in seeded melt crystallization.

spreading it to a diameter of ~0.5 cm. The temperature was kept at 130 °C for 5 min to ensure complete melting and lowered at 20 °C/min to the temperature of interest. For seeded crystallization, a seed crystal was placed on the bottom cover glass near the melt for about 3 min so that the seed achieved the temperature of the melt. A fiber loop was used to push the seed into the melt. The process of seeding and crystallization was monitored with a Nikon Optiphot2-Pol microscope equipped with a Sony digital camera. The polymorphic form of new crystals was often obvious on sight (from their colors and morphologies), but was always confirmed by Raman microscopy and PXRD. Spontaneous crystallization was performed with the same accessory but without seeds and spacers. Crystallization by vapor condensation was carried out in a sublimation apparatus in which seeds of ROY crystals were affixed to the coldfinger maintained at constant temperature by circulating tap water.

Crystal growth rates were measured for ROY polymorphs with the aid of a Nikon microscope and Linkam hot stage. ROY crystals (ca. 3 mg) were melted between two 15 mm cover glasses at 130 °C for 3–5 min and cooled to the desired temperatures of crystallization. The thickness of the liquid sample (7–10 μm) was confirmed not to affect the growth rates. Crystallization of a desired polymorph was initiated by seeds of that polymorph introduced at temperatures where no cross-nucleation occurred or by baked seeds that prevented cross-nucleation (see later). The crystal growth rate was evaluated from the advance speed of the crystal front into the melt at constant temperature. Measurements were often made with crystals that were partially remelted. This was done by placing the sample slightly off the center of the hot stage so that most of the sample still touched the heating block but a small edge of it did not. The majority of the crystals were then remelted (at 130 °C for 3–5 min), and the unmelted crystals at the edge served as seeds for the next measurement. Each crystal growth rate reported was the average of 3–4 measurements, and the standard deviation was ±5% to ±20%.

## Results and Discussion

**Spontaneous Crystallization.** ROY polymorphs that could spontaneously nucleate at room temperature (22 ± 2 °C) were Y04, YN, and ON. Y04 was the first to appear (after an induction time of ca. 30 min) (Figure 2a),<sup>19</sup> but never consumed the entire liquid. Instead, after 5–10 min of growth, it nucleated other polymorphs, which grew faster and overtook Y04 (see later for relative growth rates). The cross-nucleated polymorph was R (Figure 2b) or a previously unknown polymorph, R05 (for red, discovered in 2005) (Figure 3a). R05 is characterized by its distinct Raman spectrum ( $\nu_{CN} = 2217 \text{ cm}^{-1}$ ,  $\nu_{NH} = 3276 \text{ cm}^{-1}$ ) and X-ray diffraction pattern (Figure 3b). It converted to polymorphs Y and R in hours at room temperature or in weeks in a freezer (–12 °C). In DSC analysis (10 °C/min), it converted exothermically to Y and R (trace amount) near 45 °C, making it impossible to measure its melting point. We never observed the *spontaneous* nucleation of R and R05. That is, these polymorphs could not nucleate without the aid of Y04.

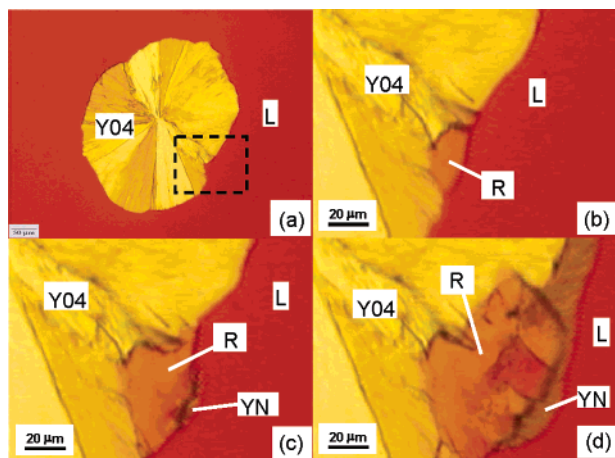
(16) Stephenson, G. A.; Borchardt, T. B.; Byrn, S. R.; Boyer, J.; Snorek, S. V.; Bunnell, C. A.; Yu, L. *J. Pharm. Sci.* **1995**, *84*, 1385.

(17) Yu, L.; Stephenson, G. A.; Mitchell, C. A.; Bunnell, C. A.; Snorek, S. V.; Bowyer, J.; Borchardt, T. B.; Stowell, J. G.; Byrn, S. R. *J. Am. Chem. Soc.* **2000**, *122*, 585.

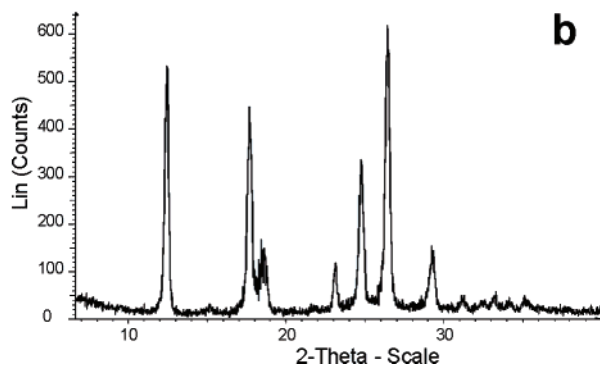
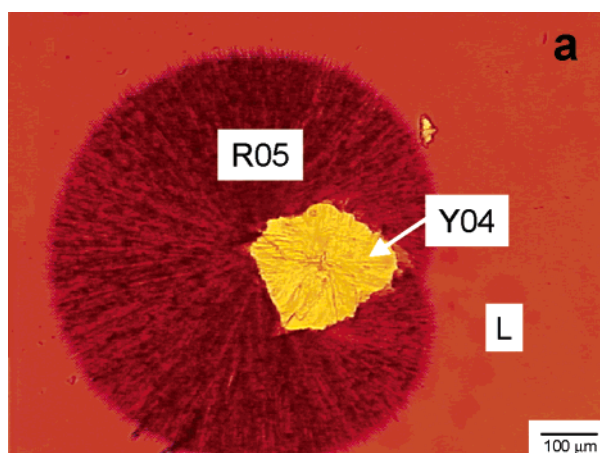
(18) Mitchell, C. A.; Yu, L.; Ward, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 10830.

(19) Chen, S.; Guzei, I. A.; Yu, L. *J. Am. Chem. Soc.* **2005**, *127*, 9881.

(20) Allen, F. H. *Acta Crystallogr.* **2002**, *B58*, 380.



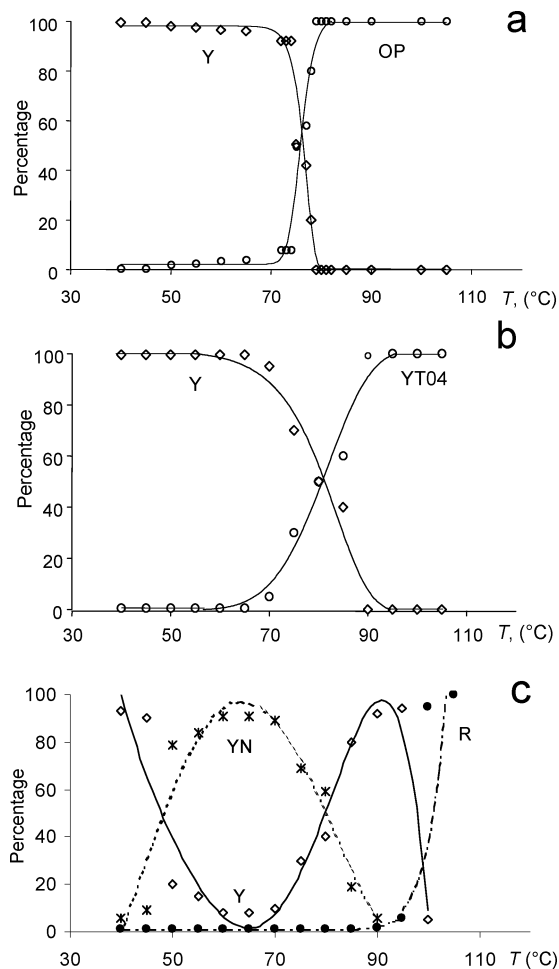
**Figure 2.** Spontaneous crystallization of ROY: (a) Y04 appeared first ( $t = 0$ ), (b) 1 min later, (c) 2 min later, (d) 3 min later. The box in (a) shows the field of view of (b)–(d). L = liquid.



**Figure 3.** (a) R05, a new polymorph of ROY, nucleated on Y04. L = liquid. (b) PXRD pattern of R05. Characteristic peaks are at 12.44°, 12.47°, 17.66°, 18.38°, 18.70°, 23.13°, 24.76°, 26.44°, 29.32°, 31.22°, and 33.19° ( $2\theta$ ). Peak intensities may be biased by the preferred orientation because needlelike crystals were analyzed without grinding to prevent polymorphic conversion.

No other polymorph (besides R and R05) was observed to nucleate on Y04. This selectivity is noteworthy considering how many polymorphs can grow from the same liquid (see later).

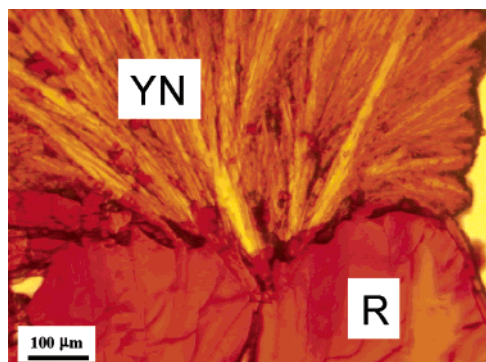
In the case of R nucleating on Y04, another yellow polymorph, YN, was observed to nucleate on R (Figure 2c,d). It is noteworthy that YN could nucleate not only on R but also spontaneously (at a slower rate than Y04). But whenever YN appeared by cross-nucleation, it did so on the surface of R. In either case, no other polymorph was found to nucleate on the



**Figure 4.** Outcome of seeded crystallization vs temperature: (a) OP seeds, (b) YT04 seeds, (c) R seeds.

surface of YN. Similar to YN, polymorph ON could nucleate spontaneously but did not cross-nucleate other polymorphs. Thus, the polymorphs differ significantly in their ability to nucleate other polymorphs: Y04 and R showed cross-nucleation, but R05, YN, and ON did not.

**Seeded Crystallization: Effect of Temperature on Cross-Nucleation.** We studied melt crystallization initiated with seeds of polymorphs Y, OP, YT04, ON, and R, all of which are stable for years at room temperature. The age of the seeds was months to years. We did not use other polymorphs as seeds because they underwent polymorphic conversion in days to weeks. Before seeding, we confirmed the polymorphic forms of the seeds by PXRD, Raman spectroscopy, and DSC. The temperature of seeding ranged from 105 °C, which is just below the melting point of lowest melting point polymorph R (106.2 °C), to 40 °C, at which no significant spontaneous crystallization occurred. With Y seeds, no cross-nucleation was observed and the new growth was Y at any temperature. With seeds of other polymorphs, however, cross-nucleation occurred and showed a strong temperature dependence. For example, above 80 °C, OP seeds yielded pure OP, but below 80 °C, Y could nucleate on the surface of OP, resulting in a mixture of OP and Y (Figure 4a). The percentages of OP and Y in the final product were estimated from the area of the sample occupied by each polymorph. The results (Figure 4a) show an increase in the percentage of Y with decreasing temperature and a well-defined



**Figure 5.** Nucleation of polymorph YN on R at 80 °C.

**Table 2.** Cross-Nucleation between ROY Polymorphs Observed in This Study

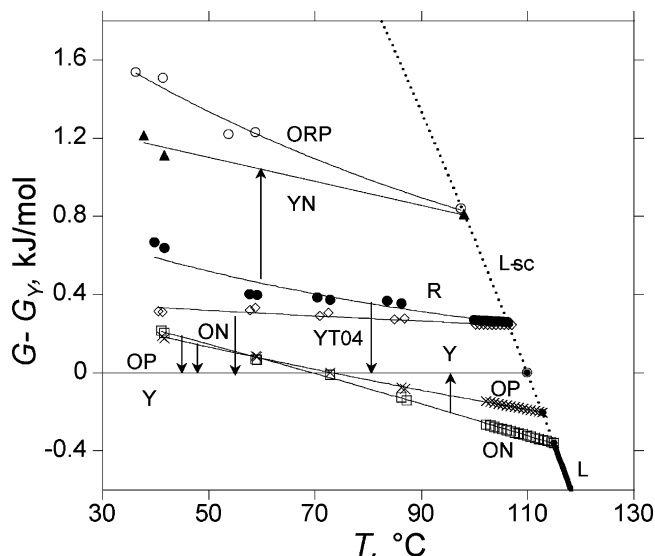
initial polymorph	new growth	remark
In Spontaneous Crystallization (22 °C)		
Y04	R	
Y04	R05	Y04, YN, and ON nucleate spontaneously
R	YN	
In Seeded Crystallization (40–105 °C)		
OP	Y	
YT04	Y	occurrence depends on <i>T</i> and the surface of the seeds
ON	Y	(see the text)
R	Y	
R	YN	

transition at 75–80 °C. Similar results were obtained with YT04 seeds, for which the transition region was 75–90 °C (Figure 4b). With ON seeds, a mixture of ON and Y was obtained at any temperature, and Y was always found to nucleate on the surface of ON.

The results with R seeds were more complicated (Figure 4c). Above 100 °C, only R crystallized. Near 95 °C, Y grew on the surface of the R seeds. Below 90 °C, YN also grew from the surface of R (Figure 5). Between 50 and 80 °C, YN was the dominant phase; however, below 50 °C, Y became the dominant phase again. Below 95 °C, no significant growth of the R seeds occurred. Because YN readily converted to R above 60 °C, the percentage of YN in Figure 4c includes both transformed and untransformed YN regions. Table 2 summarizes the cases of cross-nucleation between ROY polymorphs observed in this study.

#### Relative Stability of Polymorphs and Cross-Nucleation.

By the relative stability of polymorphs, two types of cross-nucleation may be defined: (1) a metastable polymorph nucleates on a stable polymorph and (2) a stable polymorph nucleates on a metastable polymorph. We have observed both types. As Figure 6 (the free energy–temperature diagram of ROY polymorphs constructed from melting and eutectic-melting data<sup>17,19,21,22</sup>) shows, between 40 and 70 °C, the relative stability of ROY polymorphs is Y (most stable) > ON ≈ OP > YT04 > R > YN. In this region, the stable-on-metastable cases were Y-on-R, Y-on-YT04, Y-on-ON, and Y-on-OP (down arrows in Figure 6), and the metastable-on-stable case was YN-on-R (up arrow). Above 70 °C, the stability order of ROY polymorphs is ON (most stable) > OP > Y > YT04 > R > YN. In this region, the stable-on-metastable cases were Y-on-R and Y-on-



**Figure 6.** Free energy–temperature diagram of ROY polymorphs showing stable-on-metastable (↓) and metastable-on-stable (↑) cross-nucleation between polymorphs. The diagram has been updated to include curve ORP, which could not be determined in ref 17 because of a scarcity of material. In this work, enough ORP was made by seeding a saturated solution at –12 °C.

YT04, and the metastable-on-stable cases were YN-on-R, Y-on-OP, and Y-on-ON. These results show that seeds of one polymorph can nucleate another polymorph of higher or lower thermodynamic stability. They also demonstrate once more that Ostwald's law of stages (Scheme 1)<sup>2</sup> is not a general description of nucleation in polymorphic systems. By OLS, the least stable polymorph should crystallize first, followed by transformation to the second least stable, and so on to the most stable. In spontaneous crystallization of ROY, the first appearance of Y04, the least stable of the 10 ROY polymorphs known, conforms to OLS. (The free energy of Y04 has not been determined quantitatively owing to its instability, but Y04 was found to be the least stable by polymorphic transformation.) However, OLS requires that Y04 consume the entire liquid and then transform to the next less stable polymorph (ORP). Instead, Y04 did not grow to consume the entire liquid and before transforming to another polymorph (YT04)<sup>19</sup> nucleated R, which is at least the third less stable from Y04. Moreover, OLS is inconsistent with metastable-on-stable cross-nucleation.

#### Relative Growth Rates of Polymorphs and Cross-Nucleation.

Because cross-nucleation between polymorphs is not controlled by their relative thermodynamic stability, we examined how the phenomenon depends on kinetic factors and, in particular, relative growth rates of polymorphs. First, we measured the growth rates of ROY polymorphs near room temperature. Because the room temperature fluctuated by ca. 2 °C from day to day, we used the Linkam hot stage to maintain the sample temperature at 27.0 ± 0.1 °C for all measurements. The result (Figure 7) shows the growth rates of ROY polymorphs differ by over 1 order of magnitude at 27 °C. Next we measured the growth rates of Y, OP, YT04, ON, YN, and R as a function of temperature (Figure 8). The growth rate of YN (mp 99 °C) could be measured only up to 95 °C because of polymorphic conversion. The growth rate of R could not be measured between 27 and 85 °C because YN nucleated rapidly on R in this region, but could be measured at higher or lower

(21) Yu, L. *J. Pharm. Sci.* **1995**, *84*, 966

(22) Yu, L.; Huang, J.; Jones, K. J. *J. Phys. Chem. B* **2005**, *109*, 19915.

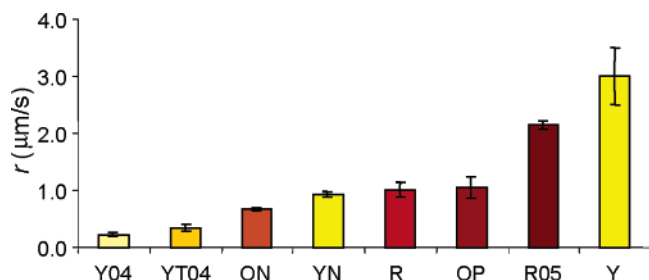


Figure 7. Growth rates of ROY polymorphs at 27 °C.

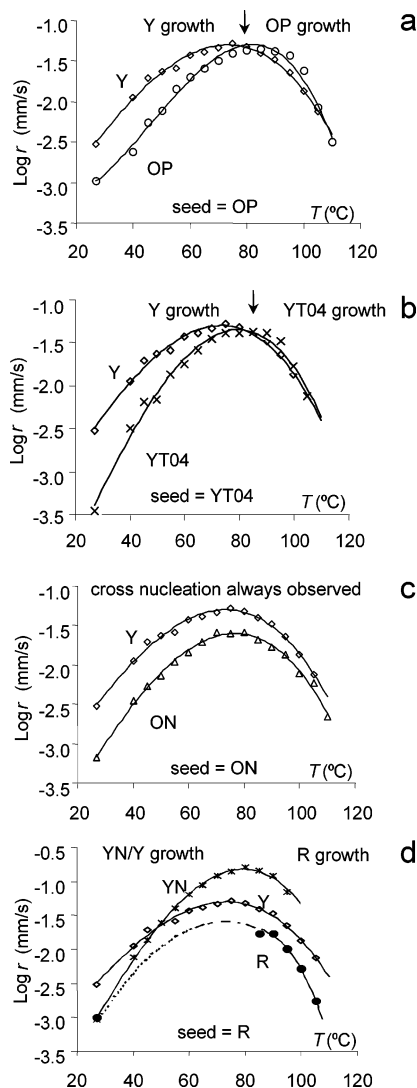


Figure 8. Growth rates of ROY polymorphs and their relation to cross-nucleation.

temperatures. The R curve in Figure 8 between 27 and 85 °C was obtained by interpolation.

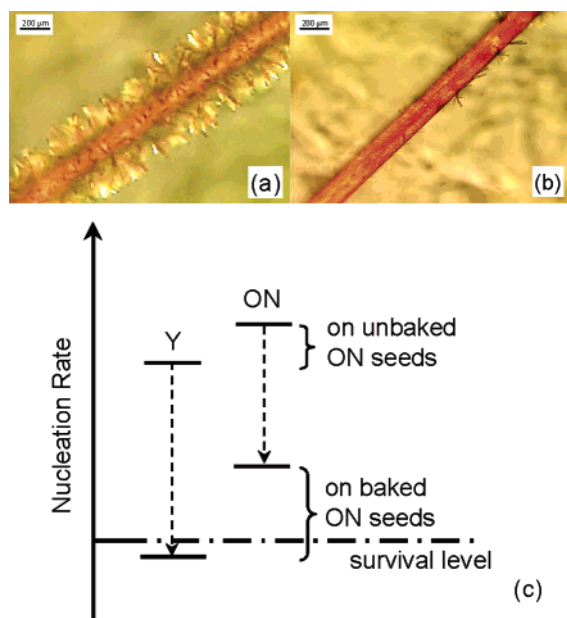
These data demonstrate that, for cross-nucleation between polymorphs to be observable, a necessary, but insufficient, condition is that the new polymorph grows faster or as fast as the initial one. In spontaneous crystallization at room temperature, all cross-nucleated polymorphs grew faster than the initial one (R and R05 on Y04) or as fast as the initial one (YN on R). In seeded crystallization, the temperature region at which Y nucleated on OP ( $T < 80$  °C, Figure 4a) coincided with that at which Y grew faster than OP (Figure 8a). Similarly, the temperature region at which Y nucleated on YT04 ( $T < 85$  °C,

Figure 4b) coincided with that at which Y grew faster than YT04 (Figure 8b). At any temperature of this study, Y grew faster than ON (Figure 8c), and Y was observed to nucleate on ON in seeded crystallization. Similarly, YN and Y grew faster than R at any temperature of this study (Figure 8d), and both were observed to nucleate on R seeds below 95 °C. However, a faster growth rate did not guarantee a new polymorph to nucleate and grow on the initial one. For example, at 27 °C, Y04 grew the slowest (Figure 7), but only R and R05 were observed to nucleate on Y04; between 50 and 90 °C, YN grew the fastest, but it nucleated only on R. Thus, for cross-nucleation to occur, the new polymorph must not only grow sufficiently fast but also nucleate sufficiently fast on the surface of the initial one.

**Seed Surface and Cross-Nucleation.** Because sufficiently fast nucleation on the initial polymorph is required for observing cross-nucleation, we studied how the surface of seed crystals affected the phenomenon. For this purpose, we annealed seed crystals by baking to reduce the number of surface defects. Polymorph ON, the most stable polymorph at high temperature (Figure 6), is suitable for this purpose. ON crystals (mp 114.8 °C) were baked at 113 °C for 20 min and immediately tested as seeds. Although unbaked ON seeds initiated the growth of Y, baked ON seeds did not and yielded only pure ON. The same effect was observed with baked OP seeds (20 min at 111 °C; mp of OP 112.7 °C): freshly baked OP seeds yielded only OP and showed no cross-nucleation. These results indicate that the observation of cross-nucleation depends not only on the relative growth rates of polymorphs but also on the rate of heterogeneous nucleation of the new polymorph. The latter, in turn, depends on the nature of the seed surface. As a result, the “transition temperatures” of cross-nucleation (Figure 4) should not be treated as intrinsic properties of polymorphs, even though they were experimentally reproducible with aged seeds.

Because Y is the most thermodynamically stable polymorph below 70 °C (Figure 6), the possibility existed that the observation of Y nucleating on other polymorphs resulted not from cross-nucleation, but from other polymorphs converting to Y on storage. This possibility was ruled out by the following evidence: (i) Analysis of unbaked seeds by PXRD, Raman spectroscopy, and DSC showed no evidence of polymorphic transformation. By these analyses, polymorphs ON, OP, YT04, and R are physically stable for years at room temperature. (ii) Unbaked seeds yielded the respective polymorphs in solution crystallization. (iii) After baking ON seeds and ensuring that they led to only new growth of ON, we “aged” the seeds at 80 °C, at which temperature ON is more stable than Y and no conversion to Y can occur (the Y/ON enantiotropic transition temperature is 70 °C; see Figure 6). After one week of aging, the ON seeds again became capable of nucleating Y. Thus, the nucleation of Y on aged ON seeds was not caused by polymorphic transformation, but by the surface modification that changed the rate of heterogeneous nucleation.

To further test the above conclusion, we compared baked and unbaked ON seeds side by side in crystallization from vapor. Figure 9 shows that the surface of an unbaked ON seed initiated many more new crystals than that of a baked ON seed. This result suggests that baking had significantly reduced the effectiveness of the seed surface to nucleate new crystals, perhaps a result of fewer surface defects. We speculate that the



**Figure 9.** Results of crystallization from vapor on the surfaces of (a) unbaked and (b) baked ON seeds. (c) Hypothesized changes of nucleation rates on baked and unbaked seeds.

nonobservation of cross-nucleation between polymorphs on baked ON seeds in melt crystallization arises from the reduction of the rate of Y nucleating on ON *that is to a greater extent* than the reduction of the nucleation rate of ON on ON (Figure 9c). Our finding suggests that cross-nucleation between polymorphs may be avoided or minimized by reconditioning the seeds.

**Polymorphic Selectivity in Cross-Nucleation.** Given the many polymorphs of ROY, it is remarkable that cross-nucleation can occur selectively between polymorphs. YN, for example, nucleated only on R under conditions of this study. Because

their crystal structures are available,<sup>17</sup> we investigated whether the YN/R selectivity is caused by lattice matching with the aid of the program GRACE.<sup>18</sup> YN grows as needles along the *a* axis, and therefore, the crystal face that contacts the substrate is likely (100). We searched for possible matches between YN(100) and other ROY polymorphs [any (*hkl*)]. We found that the lattice matches that exist between YN(100) and R are not significantly better than those between YN(100) and other polymorphs. Thus, the selective nucleation of YN on R is not caused by lattice matching or lattice matching alone. It is possible that polymorphic selectivity in cross-nucleation results from a combined effect of the different growth rates and heterogeneous nucleation rates of polymorphs. The preferred polymorph may have the optimal combination of growth rate and nucleation rate.

## Conclusions

This study found extensive and sometimes selective cross-nucleation between ROY polymorphs in spontaneous and seeded melt crystallization. Certain polymorphs could not nucleate without the aid of others. The new polymorph was found to be more or less thermodynamically stable than the initial one but to always grow faster than or as fast as the initial one. The temperature and surface characteristics of the initial polymorph affected whether cross-nucleation was observed. Our results show that crystallization in polymorphic systems is not determined solely by the initial nucleation, but also by the cross-nucleation between polymorphs and the different growth rates of polymorphs. These effects should be further studied and taken into account in the theory and control of polymorphism.

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