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Influence of Agitation and Fluid Shear on Nucleation of *m*-Hydroxybenzoic Acid Polymorphs

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ABSTRACT: The influence of agitation and fluid shear on nucleation of *m*-hydroxybenzoic acid polymorphs from 1-propanol solution has been investigated through 1160 cooling crystallization experiments. The induction time has been measured at different supersaturations and temperatures in two different crystallizer setups: small vials agitated by magnetic stir bars, for which experiments were repeated 40–80 times, and a rotating cylinder apparatus, for which each experiment was repeated five times. The nucleating polymorph has in each case been identified by FTIR spectroscopy. At high thermodynamic driving force for nucleation, only the metastable polymorph (form II) was obtained, while at low driving force both polymorphs were obtained. At equal driving force, a higher temperature resulted in a larger proportion of form I nucleations. The fluid dynamic conditions influence the induction time, as well as the polymorphic outcome. Experiments in small vials show that the agitation rate has a stronger influence on the induction time of form II compared to form I. The fraction of form I nucleations is significantly lower at intermediate agitation rates, coinciding with a reduced induction time of form II. In experiments in the rotating cylinder apparatus, the induction time is found to be inversely correlated to the shear rate. The difference in polymorphic outcome at different driving force is examined in terms of the ratio of the nucleation rates of the two polymorphs, calculated by classical nucleation theory using determined values of the pre-exponential factor and interfacial energy for each polymorph. A possible mechanism explaining the difference in the influence of fluid dynamics on the nucleation of the two polymorphs is based on differences between the two crystal structures. It is hypothesized that the layered structure of form II is comparatively more sensitive to changes in shear flow conditions than the more isotropic form I structure.

INTRODUCTION

A pure substance with the potential to crystallize as more than one crystalline phase with different ordered arrangements of molecules is said to exhibit polymorphism.¹ Polymorphs of active pharmaceutical ingredients (APIs) are of great importance to the pharmaceutical industry, since they can exhibit significantly different solubility and dissolution rates, and thereby have different bioavailability. At each given set of conditions, except for transition points, there is one thermodynamically stable polymorph, with the lowest free energy and solubility of all potential polymorphs. Which polymorph will actually crystallize first is subject to both thermodynamic and kinetic factors, however.² Thermodynamically, the stable polymorph is preferred as it will have a higher driving force for nucleation. The driving force is defined as the difference in chemical potential between the supersaturated and equilibrium states of the compound in solution, and is often approximated as $RT \ln S$, where S is the supersaturation ratio on mole fraction basis. In practice, however, the stable polymorph will often have a higher activation energy barrier for nucleation than a metastable polymorph, leading to a reduced nucleation rate.

In our previous contribution,³ a clear influence of agitation on primary nucleation in general was observed. This implies that agitation could potentially exert a different influence on the nucleation of different polymorphs, and thus possibly alter the polymorphic outcome. A few studies have explored the effect of agitation of otherwise quiescent solutions on polymorphic outcome,^{4,5} where it is suggested that postnucleation polymorphic transformation is a key mechanism involved. In this study, we provide experimental data clearly revealing that fluid dynamic conditions can have a substantial influence on the polymorphic outcome, explore the character of such an influence, and propose explanations. The compound *m*-hydroxybenzoic acid (*m*HBA) has two polymorphs with known structures,⁶ forming a monotropic system with fairly similar free energies.⁷ The solvent 1-propanol is chosen as solubility data of both polymorphs is available for this system, and as it has been demonstrated to be possible to obtain both polymorphs in phase-pure state in roughly equal proportion in repeat experiments under identical conditions in this solvent.⁷

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Repeated small-scale nucleation experiments often show a significant variation, assumed to reflect the stochastic nature of nucleation itself.^{7–13} To capture this variation and to obtain statistically valid data, a multiple-vial system has been used, consisting of several identical 20 mL vials equipped with magnetic stir bars, allowing a large number of simultaneous experiments to be conducted. Although the vial experiments can be performed in large numbers, the flow conditions are complex and poorly controlled; the fluid dynamic conditions and shear rates are very nonuniform and the shearing conditions between the stir bar and the bottom of the vial are difficult to characterize. To study a case of simpler fluid dynamics, an apparatus with two concentric cylinders, called a Taylor–Couette flow system, has also been used,³ allowing the generation of a more uniform shear stress in the solution. In each setup, the induction time for nucleation and the polymorphic outcome has been investigated at different levels of supersaturation, temperature and agitation/shear.

EXPERIMENTAL WORK

Materials. *m*-Hydroxybenzoic acid (*m*HBA, CAS Reg. No. 99-06-9, stated purity >99.0%,) shown in Figure 1, was purchased from Sigma-

Figure 1. Molecular structure of *m*HBA.

Aldrich. 1-Propanol (stated purity >99.8%) was purchased from VWR and used as received. Solutions were filtered through 5 μm PTFE membrane filters before use.

Polymorph Identification. Fourier-transform infrared (FTIR) spectroscopy was used for the identification and characterization of the polymorphs of *m*HBA. A PerkinElmer Spectrum One with an attenuated total reflectance (ATR) module equipped with a ZnSe-crystal window was used, with a scanning range of 650–2000 cm^{-1} and a resolution of 4 cm^{-1} . As discussed in a previous contribution,⁷ the two polymorphs can be easily distinguished based on comparison of their FTIR spectra, for example, from peaks at 1460 cm^{-1} , 1270 cm^{-1} , and around 740–770 cm^{-1} . As shown in Figure 2, the intensities

Figure 2. Part of FTIR spectra obtained for different mass fractions of the two polymorphs.

of unique peaks for each polymorph, for example, at 757 cm^{-1} (peak 1) for form I and at 744 cm^{-1} (peak 2) for form II, depend on the mass fraction of the respective polymorph. Using representative crystal samples, a calibration curve was constructed, Figure 3, relating the proportion of the two polymorphs to the relative intensities of these two characteristic peaks. The resulting linear relationship is used to estimate the phase purity of the material obtained in nucleation experiments to within an estimated accuracy of at least $\pm 10\%$ by weight.

Figure 3. Ratio of maximum intensities of two characteristic peaks, at 757 (peak 1) and 744 cm^{-1} (peak 2), for different mass fractions of the two polymorphs.

Nucleation Experiments in Magnetically Agitated Vials. The multiple-vial system is shown in Figure 4 a. In each run, 20 vials were

Figure 4. Setup for experiments in (a) multiple vials and (b) Taylor–Couette flow system. Reprinted with permission from our previous paper.³ Copyright 2013 American Chemical Society.

operated in parallel, held in a specially designed rack. Each vial (diameter 25 mm, height 60 mm) was filled with 10 mL of solution and furnished with a PTFE-coated magnetic stir bar (length 20 mm, thickness 6 mm, with a pivot ring in the center). The vials were placed on a submersible multipole magnetic driver unit (2mag AG). Saturated solutions were prepared in 500 mL bottles and then filtered through PTFE membrane filters (pore size 5 μm) into the vials, which were immediately capped. A flow diagram outlining the nucleation experiments is given in Figure 5. The vials filled with solution were

Figure 5. Flow diagram of nucleation experiments.

initially submerged in a temperature-controlled water bath (Julabo FP50) at a temperature 10 °C above the saturation temperature for 2 h to ensure complete dissolution. The entire rack holding the vials was then moved to a water bath at a lower temperature (T_{nucl}) to rapidly reach the desired supersaturation. A Sony HDR-XR200 high-resolution digital camcorder was used to record the progress of all vials simultaneously. The induction time, t_{ind} , is in this work taken as the time elapsing from the point when the solution is moved to the bath at T_{nucl} until nucleation can be detected by the naked eye from the recorded video. The time required to effect 95% of the total temperature change of the solution was less than 2 min, as verified by recording the temperature inside a vial. The specified temperature stability of the bath is ± 0.1 °C. Within a few seconds of the first observation of nucleation in a vial, the solution would turn clearly turbid, allowing the identification of the moment of nucleation to be done with sufficient precision from the recorded video. For this system, any slight improvement in determination of the onset of nucleation that could possibly be obtained through using more advanced detection methods would have a negligible impact on individual induction times, and the reduction in the uncertainty in average induction times would be negligible compared to the stochastic variation observed among repeat experiments. Moreover, application of more advanced methods would make each measurement more laborious, and necessitate sacrificing some statistical validity.

As soon as sufficient crystalline material had precipitated in a vial (between 8 and 14 min after nucleation was observed), the contents were filtered using Munktell grade 00A filter paper and dried in a ventilated fume hood. FTIR spectroscopy was then used to identify the polymorph. All solution concentrations were verified gravimetrically.⁷ The nucleation experiments were conducted at several different temperatures, agitation rates and levels of supersaturation. For each set of conditions a total of 40 experiments were performed (i.e., 2 times 20 parallel vials).

To standardize solution pretreatment, the solutions were filtered through 0.2 μm PTFE syringe filters during the filling of each vial. Each vial was filled with an equal amount of solution. All the vials were submerged in the higher-temperature water bath for the same time period (2 h) in order to avoid differences in solution history.^{10,14,15} The experiments were conducted at a sufficiently low supersaturation so as to ensure that induction times were long enough for the time required for cooling the vials to the target temperature to be negligible.

Nucleation Experiments in Taylor–Couette Flow System. The Taylor–Couette flow system used, described in detail elsewhere,³ is shown in Figure 4b. The setup features a sealed, cylindrical glass shell (diameter 50 mm, height 150 mm) with a rotating inner cylinder. The gap between the two cylinders is 5 mm. The vessel was completely filled with solution (approximately 150 mL) and sealed. The entire vessel except for the topmost part was then submerged in a temperature-controlled water bath. The nucleation experiments were carried out according to the flow diagram in Figure 5, and the point of nucleation was detected in the same way as in the vial experiments. As soon as sufficient crystalline material had precipitated, 10 mL of the solution together with suspended crystals were sampled using a syringe and filtered through Munktell grade 00A filter paper. The vessel was then topped up with 10 mL of fresh solution of the same concentration and replaced in the higher-temperature water bath, and the steps in Figure 5 repeated five times for each rotation rate. Experiments were carried out at four different rotation rates, namely, 100, 200, 300, and 400 rpm. Similar precautions to ensure reproducibility within the stochastic variability were undertaken as for the vial experiments. It was shown in a previous study³ that the time required to effect 95% of the total temperature change under conditions similar to those of the present work is less than 6 min, and in 9 min the target temperature has been reached to within ± 0.2 °C.

Influence of Postnucleation Polymorphic Transformation. In this study, all solutions were sampled and filtered within 8–14 min of observed nucleation. In most experiments, the nucleation temperature was low, the highest recorded nucleation temperature is 30 °C, and the rate of transformation of form II crystals into the stable form I should be negligible.⁷ To verify that postnucleation polymorphic trans-

formation was not an influential factor in this study, a group of control experiments were carried out using the vial setup. A set of nucleation experiments were repeated at exactly the same conditions, but the solids were filtered at different times after nucleation. Figure 6 shows

Figure 6. Influence of the residence time of solids in solution on the polymorphic outcome, for vial experiments with $T_{\text{sat}} = 55$ °C, $T_{\text{nucl}} = 25$ °C, and $N = 800$ rpm. Bars indicate 95% confidence intervals calculated with the Wilson equation.^{16,17}

the proportion of the two polymorphs in three groups of experiments where the solid has been isolated from the solution after different times. Each group consists of 40 experiments. As there is statistically no significant difference in the polymorphic outcome between the three groups of experiments, this indicates that postnucleation polymorph transformation should have a negligible influence on the experimental results.

RESULTS AND EVALUATION

Twenty-eight series of nucleation experiments have been performed, with each series consisting of at least 40 repeat experiments in vials and 5 repeat experiments in the Taylor–Couette flow system, amounting to 1160 experiments in total. Experimental details are summarized in Tables 1 and 2. Series 1–20 were conducted in vials. In these experiments, the influence of the driving force ($RT \ln S$), nucleation temperature and rotation rate on the induction time and the polymorphic outcome has been systematically studied. Series 21–28 were conducted in the Taylor–Couette flow system. Series 21–24 and 25–28 were carried out under the same conditions as series 2–5 and series 9–12, respectively.

It should be clearly recognized that in each individual crystallization experiment only one polymorph crystallized, to the limit of detection using FTIR and the calibration curve shown in Figure 3. However, the outcome of repeat experiments performed at the same conditions would often differ with respect to the crystallizing polymorph, and this is quantified as a fraction of experiments resulting in each respective polymorph. The fact that we always obtain either polymorph in pure form suggests that, following primary nucleation of a given polymorph, the growth of crystals of that form is sufficiently fast to consume the supersaturation and prevent primary nucleation of the other polymorph.

Influence of Supersaturation and Temperature in Vial Experiments. Figure 7 shows cumulative induction time distributions in the vial experiments at an agitation rate of 100 rpm are shown in Figure 7 for selected values of the driving force of nucleation ($RT \ln S$, expressed with respect to the solubility of form I). As expected, with decreasing driving force the induction time increases. The coefficient of variation (CV) is about the same for all distributions, ranging between 0.27 and 0.31.

Table 1. Details, Polymorphic Outcome, and Average Induction Times for Vial Experiments

series no.	$RT \ln S$ [$J \text{ mol}^{-1}$]	T_{nuc} [$^{\circ}\text{C}$]	no. of exp.	N [rpm]	form I		form II	
					fraction [%]	t_{ind} [min]	fraction [%]	t_{ind} [min]
1	798	30	40	100	90.0	65.00	10.0	81.00
2	852	25	80	100	40.0	34.95	60.0	50.15
3	852	25	80	200	17.5	36.65	82.5	32.89
4	852	25	80	400	13.8	35.00	86.2	29.89
5	852	25	80	800	50.0	49.88	50.0	62.63
6	851	22	40	100	20.0	41.02	80.0	50.30
7	936	30	40	100	60.0	20.45	40.0	27.06
8	941	28	40	100	40.0	24.00	60.0	30.70
9	940	25	80	100	37.5	33.85	62.5	42.15
10	940	25	80	200	15.0	34.45	85.0	28.89
11	940	25	80	400	15.0	39.90	85.0	30.84
12	940	25	80	800	47.5	43.28	52.5	50.31
13	933	19	40	100	15.0	30.90	85.0	35.40
14	941	11	40	100	0.0		100.0	38.41
15	934	2	40	100	0.0		100.0	43.00
16	1024	22	40	100	0.0		100.0	13.05
17	1024	22	40	200	0.0		100.0	13.60
18	1024	22	40	400	0.0		100.0	13.05
19	1024	22	40	800	0.0		100.0	14.20
20	1117	18	40	100	0.0		100.0	14.10

Table 2. Details, Polymorphic Outcome, and Average Induction Times for Taylor–Couette Experiments

series no.	$RT \ln S$ [$J \text{ mol}^{-1}$]	T_{nuc} [$^{\circ}\text{C}$]	no. of exp.	N [rpm]	form I		form II	
					fraction [%]	t_{ind} [min]	fraction [%]	t_{ind} [min]
21	852	25	5	100	40.0	57.50	60.0	68.33
22	852	25	5	200	0.0		100.0	44.60
23	852	25	5	300	20.0	40.00	80.0	40.25
24	852	25	5	400	0.0		100.0	39.80
25	940	25	5	100	40.0	45.00	60.0	50.67
26	940	25	5	200	0.0		100.0	32.80
27	940	25	5	300	0.0		100.0	30.80
28	940	25	5	400	0.0		100.0	26.80

Figure 7. Cumulative induction time distributions in vial experiments at different driving force (with respect to form I), $N = 100$ rpm.

Figure 8 shows the fraction of all nucleations resulting in form I in vial experiments at an agitation rate of 100 rpm at different driving force and nucleation temperature. Each data point represents the mean of 40 experiments. At higher driving force only form II was obtained, while at lower driving force both polymorphs were obtained. There is an overall trend of

Figure 8. Influence of nucleation driving force on polymorphic outcome, for vial experiments at different driving force (with respect to form I) and nucleation temperature, $N = 100$ rpm. Bars indicate 95% confidence intervals calculated with the Wilson equation.^{16,17}

increasing proportion of form I nucleations with decreasing driving force. However, comparing experiments at similar driving force but different nucleation temperature shows that

the effect of temperature on polymorphic outcome is significant.

Figure 9 shows the fraction of nucleations resulting in form I and the average induction time for experiments with different

Figure 9. Influence of nucleation temperature on polymorphic outcome and average induction time, for vial experiments at approximately equal driving force, 933–941 J/mol (with respect to form I), $N = 100$ rpm. Bars indicate 95% confidence intervals.

nucleation temperature carried out at almost constant driving force with respect to form I of 933–941 J/mol. The average induction time decreases slightly with increasing temperature. However, it is clearly shown that a higher temperature results in a higher proportion of form I when the driving force is kept constant. The solubility curves of the two polymorphs in 1-propanol, when expressed as the natural logarithm of the mole fraction vs temperature, are almost parallel,⁷ and it follows from this that if the driving force with respect to form I is kept constant for different nucleation temperatures, a constant driving force with respect to form II is also maintained. Hence, the reason for the difference in polymorphic outcome must necessarily be kinetic in origin.

Influence of Agitation Rate in Vial Experiments. In all the vial experiments carried out under conditions of high driving force ($RT \ln S_1 > 1000$ J/mol), only form II was obtained. For these experiments, the influence of agitation rate on the induction time is shown in Figure 10. Each point in the figure represents the average of 40 experiments (i.e., 2 times 20 parallel vials). Because of the high driving force induction times are fairly short, and show no dependence on the agitation rate.

In the vial experiments carried out under conditions of lower driving force ($RT \ln S_1 < 1000$ J/mol), both polymorphs were obtained. The influence of agitation rate on the induction time

Figure 10. Influence of agitation rate on average induction time, for vial experiments at constant nucleation temperature of 22 °C and a driving force of 1024 J/mol (with respect to form I). Bars indicate 95% confidence intervals.

of the two polymorphs is shown in Figure 11a and b for two different values of the driving force. Each point in the figures represents the average induction time of that particular polymorph over a total of 80 experiments (4 times 20 parallel vials) at a given agitation rate. Apparently, the agitation rate does not influence the induction time of the two polymorphs in the same way. For nucleation of form I there is a weak but steady increase in induction time with increasing agitation rate, while the trend observed for the induction time of form II with increasing agitation rate is initially decreasing and then increasing again at higher agitation rates. The behavior observed for nucleation of form II is reminiscent of the results obtained for butyl paraben³ as well as of the findings of Mullin and Raven.^{18,19} Altogether, this dependence of the ratio of induction times of the two polymorphs on agitation rate indicates that nucleation of form II should be comparatively more favored at intermediate agitation rates.

For the same sets of experiments as in Figure 11, the proportion of form I nucleations at each agitation rate is shown in Figure 12. A minimum in the fraction of form I nucleations is indeed observed at intermediate agitation rates (200 and 400 rpm) for both supersaturations.

Taylor–Couette Experiments. In a previous contribution,³ we illustrated that, for the range of rotation rates used in these experiments (100–400 rpm), the influence of Taylor vortices is quite limited. This allows shear rates to be calculated using the Navier–Stokes equation, and it was shown that the shear rate in the Taylor–Couette flow is reasonably uniform. In Figure 13, the average induction time is plotted versus the average shear rate for all experimental conditions. Each point represents an average of five repeat experiments. The figure suggests that the induction time is inversely correlated to the shear rate in the solution. This trend was observed for both investigated levels of supersaturation, with shorter induction times obtained at higher supersaturation. These results concur with what was found for butyl paraben-ethanol solutions.³

In the present work, as well as in the previous study on butyl paraben, it is found that for experiments at the same rotation rate, temperature and driving force, the average induction time obtained in the Taylor–Couette experiments is longer than that of the vial experiments. This is noteworthy since the liquid volume in the Taylor–Couette experiments is 15 times larger, and it has been suggested that, given otherwise identical conditions, the probability of nucleation event occurring within a certain time should be proportional to the solution volume.²⁰ An approximate comparison of the fluid dynamics of the two types of experiments is given in Table 3. The energy dissipation rate for the Taylor–Couette device has been calculated using Sinevic's correlation:^{21,22}

$$\varepsilon = \frac{\pi L r^4 \omega^3}{V} 0.8 \left(\frac{d}{r} \right)^{0.35} \left(\frac{\omega r d}{\nu} \right)^{-0.53} \quad (1)$$

where L , r , and ω are the length radius and angular velocity of the inner cylinder; V is the volume of the liquid; d is the distance of the gap between inner cylinder and the wall, and ν is the kinematic viscosity. For the Taylor–Couette experiments, average and maximum shear rates are calculated in our previous study.³ Treating the agitation in the vials as if the stir bar is suspended from a rotating shaft, the energy transferred by the stir bar and the average shear rate in the vial experiments have been calculated in the previous study.³ A rough estimate of the

Figure 11. Influence of agitation rate on average induction time for nucleation of each polymorph, for vial experiments with a nucleation temperature of 25 °C and a driving force of (a) 852 and (b) 940 J/mol (with respect to form I). Bars indicate 95% confidence intervals.

Figure 12. Influence of agitation rate on polymorphic outcome, for vial experiments with a nucleation temperature of 25 °C and a driving force (with respect to form I) of 852 (hollow triangles) and 940 J/mol (solid circles). Bars indicate 95% confidence intervals.

Figure 13. Influence of shear rate on average induction time (regardless of polymorph), for Taylor–Couette experiments with a nucleation temperature of 25 °C and a driving force (with respect to form I) of 852 (hollow triangles) and 940 J/mol (solid circles). Bars indicate 95% confidence intervals.

Table 3. Comparison of the Fluid Dynamics of the Vial and Taylor–Couette Experiments

vial experiments				Taylor–Couette experiments			
N [rpm]	ϵ [Wkg ⁻¹]	γ_{av} [s ⁻¹]	γ_{max} [s ⁻¹]	N [rpm]	ϵ [Wkg ⁻¹]	γ_{av} [s ⁻¹]	γ_{max} [s ⁻¹]
100	0.003	12.4	250	100	0.007	47.7	41.9
200	0.018	45.6	500	200	0.040	95.4	83.8
				300	0.108	143	125
400	0.129	122	1000	400	0.219	190	167
800	0.887	320	2000				

maximum fluid shear rate in the vial experiments is 150N, as for a turbine-stirred tank.²³

Although the mode of supplying agitation in the two setups are fundamentally different and the fluid dynamics of the stir bar agitation are more difficult to characterize, Table 3 suggests more powerful average fluid dynamics in the Taylor–Couette experiments; only the maximum shear rates, found in a fairly small part of the volume, are higher in the vial experiments. Altogether, based on comparing volumes and bulk fluid dynamics, the induction times obtained in the vial experiments are surprisingly short. It is our hypothesis that the contact between the stir bar and the vial results in high local shear rates, promoting nucleation.

Figure 14 shows the polymorphic outcome in the Taylor–Couette experiments. The highest proportion of form I was

Figure 14. Influence of rotation rate on polymorphic outcome, for Taylor–Couette experiments (out of 5 experiments) with a nucleation temperature of 25 °C and a driving force (with respect to form I) of 852 (hollow triangles) and 940 J/mol (circles).

obtained at the lowest rotation rate, while form II nucleations dominate at higher rotation rates. This trend corresponds qualitatively with that observed for the vial experiments with the same nucleation temperature and supersaturation level, for agitation rates between 100–400 rpm.

ANALYSIS AND DISCUSSION

Nucleation Kinetics and Influence of Supersaturation on Polymorphic Outcome. It is generally assumed that a solution, which is supersaturated with respect to a number of polymorphs will contain subcritical clusters, in turn containing structural features promoting nucleation of these respective polymorphs.²⁴ According to the classical nucleation theory, the rate of primary nucleation of a given solid phase can be expressed as¹

$$J = A_n \exp\left(-\frac{16\pi\sigma^3 v_m^2}{3k^3 T^3 \ln^2 S}\right) \quad (2)$$

if the nucleus is assumed to be spherical. The exponential term represents a free energy barrier for the formation of a stable nucleus, while the pre-exponential factor A_n can be considered as a kinetic parameter or a rate constant. If the induction time is assumed to be inversely proportional to the nucleation rate, eq 2 can be expressed as

$$\ln t_{\text{ind}} = -\ln A + \frac{B}{T^3 \ln^2 S} \quad (3)$$

where A is proportional to the pre-exponential factor A_n and B equals $16\pi\sigma^3 v_m^2 / 3k^3$. It is occasionally assumed that $A = VA_n$ where V denotes the volume of the solution. By plotting the mean values of $\ln t_{\text{ind}}$ versus $1/(T^3 \ln^2 S)$ for vial experiments with an agitation rate of 100 rpm carried out at different supersaturations and temperatures (data from series 1–2, 6–9, 13–16, and 20), and fitting a linear function to the data, Figure 15, the crystal-solution interfacial energy of each polymorph can be estimated from the slope of the respective line. Resulting values are given in Table 4.

Figure 15. Regression according to eq 3 for vial experiments, $N = 100$ rpm.

Table 4. Slope and Intercept Obtained from Figure 15 and Resulting Values of the Interfacial Energy of the Two Polymorphs

polymorph	$-\ln A$ [s]	B [K^3]	σ [mJ m^{-2}]
form I	5.37	7.68×10^6	3.69
form II	6.03	2.16×10^6	2.42

The table shows that the interfacial energy is lower for the metastable polymorph, as expected. However, the data also shows that the pre-exponential factor for the stable polymorph is higher. On the basis of certain assumptions,^{25,26} the pre-exponential factor for primary homogeneous nucleation of spherical particles can be expressed as

$$A_n = \frac{DC \ln S}{v_{m,\text{solvent}}} \sqrt{\frac{kT}{\sigma}} \quad (4)$$

where D is the diffusion coefficient, C is the concentration of solution, and $v_{m,\text{solvent}}$ is the molecular volume of the solvent. Among the parameters in eq 4, only the supersaturation S and the interfacial energy σ will be different for two polymorphs in a given solution. Therefore, the ratio of the pre-exponential factors between the two polymorphs can be expressed as

$$\frac{A_I}{A_{II}} = \frac{A_{n,I}}{A_{n,II}} = \frac{\ln S_I}{\ln S_{II}} \sqrt{\frac{\sigma_{II}}{\sigma_I}} \quad (5)$$

At all evaluated experimental conditions the value of $\ln S_I / \ln S_{II}$ is in the range 1.4–2.0. By inserting the values of σ from Table 4, we get a range of values of $A_{n,I} / A_{n,II}$ of 1.13–1.62. By directly taking the values of A given in Table 4 we obtain $A_I / A_{II} = 1.93$, which is surprisingly close given the uncertainty involved in estimating the intercepts of the lines in Figure 15. On the basis of eq 2, the ratio of nucleation rates of the two polymorphs can be written

$$\frac{J_I}{J_{II}} = \frac{A_{n,I}}{A_{n,II}} \exp\left(-\frac{16\pi v_m^2}{3k^3 T^3} \left(\frac{\sigma_I^3}{\ln^2 S_I} - \frac{\sigma_{II}^3}{\ln^2 S_{II}}\right)\right) \quad (6)$$

By substituting the values in Table 4 and using the solubility data from previous work,⁷ we can estimate the ratio of nucleation rates of the two polymorphs at different solution concentrations and nucleation temperatures, Figure 16. For a

Figure 16. Ratio of nucleation rates of form I: form II vs solution concentration for vial experiments at two nucleation temperatures, 22 °C (circles) and 25 °C (hollow triangles).

certain nucleation temperature, as the solution concentration increases, J_I / J_{II} decreases from a value above unity to a value below unity. This indicates that the nucleation rate of form I is higher than that of form II at lower driving force but becomes gradually lower at increasing driving force. This is in agreement with our experimental results with respect to the polymorphic outcome in the vial experiments, Figure 8, namely, that form II is the dominant product at higher driving force while form I dominates at lower driving force.

Influence of Agitation and Shear. In the Taylor–Couette experiments, with allowance for the limited amount of data, the induction times of both polymorphs decrease with increasing agitation rate, similar to what was found in our previous work on butyl paraben.³ In the vial experiments, the outcome is more complex. Within the range studied the nucleation of form I is not strongly influenced by the agitation rate, and contrary to our previous findings for butyl paraben: there is a steadily increasing induction time with increasing agitation rate with no preceding minimum. The influence of agitation rate on the nucleation of form II is more in accordance with that seen for butyl paraben. With increasing agitation rate the induction time first decreases, reaches a minimum at 200–400 rpm and then increases again. It is worth noting that this minimum occurs at about the same rotation rate as for butyl paraben, and that in the Taylor–Couette experiments both compounds show the same steady decay in induction time with increasing rotation rate.

It was observed during the analysis of the vial experiments that the mode of motion of the stir bar depends on the

agitation rate. To further investigate this aspect of mixing in the vials, high resolution video recordings at 25 fps were examined and the motion of the stir bar analyzed frame-by-frame. At low agitation rates (100–400 rpm), the behavior of the stir bar can be considered as a superposition of two types of motion: a spinning motion (Figure 17a) and a repeating sliding motion

Figure 17. Different types of stir bar motion observed in the vial experiments.

from the center to the periphery and back (Figure 17b). At 100 rpm, the stir bar spends comparatively more time near the wall of the vial, while at 200 and 400 rpm, the total motion is more regular in the center, and dominated by spinning. However, at the highest agitation rate (800 rpm) the stir bar behaves very differently, sliding along the vial wall (Figure 17c) at the specified rate of revolution. Which kind of motion will be more conducive to nucleation is uncertain, because both modes contain components of friction against the bottom, collision with the walls and fluid shear, and the exact mechanisms by which these components influence nucleation are not clear. However, the transition from one behavior to another at about 400 rpm does correspond to the observed minimum in induction time versus agitation rate, for *m*HBA form II (Figure 11) as well as for butyl paraben,³ and accordingly could possibly explain these minima.

On the basis of the observed difference between the two polymorphs in the response of the induction time to changes in the agitation rate, Figure 11, it may be concluded that agitation at rates of 200 and 400 rpm promotes nucleation of form II more than form I. This in turn explains the higher proportion of form II at these two agitation rates (Figure 12). In our previous paper it is suggested that the most plausible mechanism explaining the influence of agitation on primary

nucleation involves the aggregation of clusters in solution, which is expected to be enhanced by increased agitation.³ In the case of *m*-hydroxybenzoic acid, we expect aggregation of each species of cluster to be enhanced by agitation, but the effect may very well depend on specific properties of these clusters and the crystal structure to be formed. In particular, we may distinguish between two related properties of the crystal structure. First, if clusters may in fact be considered crystalline particles of size insufficient for them to be thermodynamically stable, in accordance with the classical nucleation theory, we may assume that, in analogy with macroscopic crystals, clusters will have anisotropic shapes. Aggregation of clusters with a needle- or plate-like shape would be comparatively more favored by increased fluid shear than for more isotropically shaped clusters, as the former would experience a larger orientation effect under shear. Second, the presence of more or less well-defined crystallographic slip planes in a cluster, whether fully crystalline or, in accordance with a two-step mechanism,²⁷ not fully crystalline, could hypothetically cause it to be more sensitive to shear stress, by increasing the mobility of molecular layers.

Figure 18 illustrates the main molecular packing and hydrogen bonding features of the two structures of *m*-hydroxybenzoic acid. The form I structure consists of centrosymmetric carboxylic acid dimers, well-documented²⁸ to be a common synthon in similar systems, occurring both in structures and solutions. The dimers are packed in a herringbone pattern at a considerable angle out of the crystallographic *bc* plane as well as with respect to each other, and interacting with other dimers through chains of hydroxyl–hydroxyl interactions. The structure of form II is fundamentally different, with molecules ordered in straight, parallel pairs of chains along the crystallographic *c*-direction, with hydrogen bonds between carboxyl and hydroxyl groups. The chains are stacked with relatively small offset and difference in interplanar angle, resulting in a structure that is somewhat layered in the crystallographic *b* direction. Along the *a* direction, the double chains are ordered in straight layers with interactions only between nonpolar parts, and very limited steric interpenetration, suggesting a potential crystallographic slip plane (as indicated in Figure 18).

Figure 18. Molecular packing and hydrogen bonding in the structures of form I and form II, with the crystallographic *bc* slip plane in the form II structure shown in gray.

Table 5. Comparison of Calculated Lattice Energies with Experimental Sublimation Enthalpy and RMSD of Changes in Unit Cell Parameters upon Geometry Optimization, for Two Force Fields

polymorph	CSD refcode	space group	$\Delta_{\text{sub}}H_{0\text{K}}$ [kJ mol ⁻¹]	PcFF			KTHUL		
				E_{latt} [kJ mol ⁻¹]	ΔE_{latt} [%]	RMSD _{cell} [%]	E_{latt} [kJ mol ⁻¹]	ΔE_{latt} [%]	RMSD _{cell} [%]
form I	BIDLOP ⁶	$P2_1/b$	-129.5 ³⁵	-131.6	1.6	1.5	-117.8	9.0	1.2
form II	BIDLOP01 ⁶	$Pna2_1$		-133.5		3.3	-124.7		1.3

Figure 19. Center-to-center intermolecular interactions (green dashed lines, labels pertaining to Table 6) and hydrogen bonds (red dashed lines) in the structures of form I and II. Left-hand images show interactions in the molecular plane; right-hand images show interactions out of the molecular plane.

Table 6. Chief Intermolecular Interactions, with the Total Energy (E_{tot}) of the Interaction Together with the Electrostatic (E_e) and Dispersive (E_{vdw}) Contributions, Calculated with Two Force Fields, for the Two Polymorphs

interaction	PcFF			KTHUL		
	E_{tot} [kJ mol ⁻¹]	E_e [kJ mol ⁻¹]	E_{vdw} [kJ mol ⁻¹]	E_{tot} [kJ mol ⁻¹]	E_e [kJ mol ⁻¹]	E_{vdw} [kJ mol ⁻¹]
form I						
A	-52.72	-60.38	7.65	-66.76	-94.39	27.63
B	-25.26	-6.74	-18.53	-16.68	-3.18	-13.50
C	-24.84	-24.85	0.01	-30.60	-29.42	-1.18
D	-17.99	-1.30	-16.70	-14.55	-2.76	-11.79
E	-16.35	-0.67	-15.68	-18.51	-2.26	-16.25
F	-8.95	-1.46	-7.48	-6.95	-0.92	-6.03
G	-7.03	-1.17	-5.86	-5.16	-1.67	-3.49
H	-3.16	1.42	-4.58	-2.29	0.35	-2.64
form II						
I	-34.65	-33.18	-1.47	-41.03	-46.65	5.62
J	-26.43	1.76	-28.18	-21.68	3.21	-24.88
K	-24.95	-23.85	-1.10	-33.73	-38.70	4.97
L	-7.69	-0.96	-6.72	-6.49	-1.44	-5.05
M	-9.70	1.63	-11.33	-11.49	-2.05	-9.44
N	-3.86	-3.85	-0.01	-2.51	-0.52	-1.99

To quantify the differences in intermolecular interactions between the two structures, the systems were modeled with molecular mechanics using the software package Materials Studio 5.0 (Accelrys). Two force fields were used: (i) Pcff,²⁹ a generic force field parametrized for organic molecules, was used together with built-in point charges; a combination shown to work adequately for similar systems,³⁰ and (ii) an ad-hoc parametrized force field here termed KTHUL, based on the General Amber force field,³¹ with *exp*-6 parameters taken from the early work of Williams,^{32,33} and point charges assigned by fitting to the electrostatic potential of the single molecule optimized in vacuum at the DFT/HCTH-level.³⁴ Torsional parameters as well as some *exp*-6 parameters in the KTHUL force field have been reassigned based on ab initio calculations on a set of carboxylic acid molecules.

Missing hydrogen atoms were manually added to the form II structure, and the molecular geometry and packing of the two crystal structures were optimized. Lattice energies were calculated by subtracting the total energy of the single molecule optimized in vacuum from the total energy of the crystal structure, and compared to experimentally determined enthalpies of sublimation,³⁵ adjusted to compensate for the temperature difference by approximating the heat capacity difference between the solid and the gas as $2RT$.³⁶ Reduced unit cell parameters of the optimized structures were compared to experimental values. The results are given in Table 5. The errors in lattice energy for both force fields are within the uncertainty margin, given the approximations and uncertainties in experimental data.^{30,36,37} The changes to cell parameters resulting from optimization with the KTHUL force field are smaller than for Pcff, but both are within acceptable limits.

Crystal graphs of the optimized structures were constructed, defined to encompass all pairwise intermolecular interactions between a center molecule and its nearest shell of neighbors, all of which exceed $0.5RT$ in magnitude (1.25 kJ/mol at room temperature). The interactions are shown in Figure 19 and quantified in Table 6. For both structures, a limited number of short-distance interactions dominate the total lattice energy. With respect to the order of energies and the relative magnitudes of electrostatic and dispersive contributions, the two force fields show qualitatively similar results. Many interactions are mainly dispersive, resulting from a general close-packing and should not be very dependent on exact molecular orientation. In the case of form I, the dimer interaction A, completely electrostatic in origin, is dominant. The all-hydroxyl hydrogen bond chain C, running at an angle of about 20° out of the molecular plane, is a second considerable, mainly electrostatic interaction in the form I structure. In the form II structure, the two hydrogen bond interactions I and K, both in the molecular plane, are the only major electrostatic interactions. Apart from these, the short-distance, out-of-plane J interaction acting between stacked molecular chains in the *b* direction, is the only strong interaction in the form II structure.

The sum total is that the structure of form II is considerably less isotropic than that of form I, with respect to the total lattice enthalpy as well as directional electrostatic interactions. The form I structure is not layered, and the dimers are connected by strong hydrogen bond chains in three dimensions. In contrast, the structure of form II has rather well-defined layers, held together by weak, van der Waals-dominated L interactions. The calculations with both force fields substantiate the classification as a crystallographic slip plane. Furthermore, as the attachment energy across the slip plane will be low, the {200}-face is

expected to grow slowly,³⁸ and hence, barring strong solvent effects, dominate the morphology. Altogether, these fundamental structural differences should make clusters with a form-II-like structure more sensitive to shear stress and to changes in flow conditions, which in turn could be one reason why agitation has a stronger influence on nucleation of form II than form I.

CONCLUSIONS

The polymorphic outcome in nucleation experiments of *m*-hydroxybenzoic acid in solutions of 1-propanol is shown to depend on both the supersaturation and the nucleation temperature. With increased driving force for nucleation the proportion of form I nucleations increases, and at constant driving force an increased nucleation temperature results in a higher proportion of form I. It is shown how the difference in polymorphic outcome at different driving forces can be explained by the ratio of nucleation rates of the two polymorphs.

The two polymorphs are shown to exhibit a different influence of the agitation rate on the induction time. The agitation rate is observed to have a stronger influence on the induction time of form II compared to form I. The trend of changing induction time of form II with agitation rate corresponds qualitatively to previously reported results for the compound butyl paraben,³ that is, the induction time is first reduced as the agitation rate increases, then to rise again from a minimum value as the agitation rate is increased further. Experiments in a Taylor–Couette flow system show that the induction time is inversely related to the fluid shear rate in the regime investigated. The agitation rate is also found to influence the polymorphic outcome. In repeat experiments in vials, the proportion of form I decreases at intermediate agitation rates (200 and 400 rpm), accompanied by a comparatively large reduction in the induction time of form II. In repeat Taylor–Couette flow system experiments, the proportion of form I decreases with increasing rotation rate, from a maximum at the lowest rotation rate. On the basis of molecular modeling using two force fields, it is hypothesized that the observed difference in the effect of agitation on the two polymorphs stems from fundamental differences in the crystal structures, where nucleation of the straight, parallel chain structure of the form II structure should be comparatively more sensitive to changes in shear flow conditions.

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Notes

The authors declare no competing financial interest.

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NOTATION

A constant

A_n pre-exponential factor in nucleation rate equation

B constant
C concentration
d distance between inner and outer cylinders in TC cell
D diffusivity
E energy
J steady-state nucleation rate
k Boltzmann constant
L length of inner cylinder in TC cell
m mass
M molecular weight
N rotation rate
r radius of inner cylinder in TC cell
R gas constant
S supersaturation ratio
T temperature
V solution volume
t time
 γ shear rate
 ϵ energy dissipation rate
 ν kinematic viscosity
 v_m molecular volume
 σ interfacial energy
 ω angular velocity of inner cylinder in TC cell

Subscripts

av average
cell unit cell
e electrostatic
I form I
II form II
ind induction
latt lattice
max maximum
nucl nucleation
sat saturation
sub sublimation
tot total
vdw dispersive

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