# Influence of Solution Thermal and Structural History on the Nucleation of *m*-Hydroxybenzoic Acid Polymorphs

*Fredrik L. Nordström[†], Michael Svärd[‡], Baldur Malmberg[‡] and Åke C. Rasmuson[*‡§]*

[†)] Abbott Laboratories, North Chicago, IL, USA

[‡)] Department of Chemical Engineering and Technology, KTH Royal Institute of Technology, Stockholm, Sweden

[§)] Department of Chemical and Environmental Science, Solid State Pharmaceutical Cluster, Materials and Surface Science Institute, University of Limerick, Limerick, Ireland

[*)] To whom correspondence should be addressed. E-mail: rasmuson@ket.kth.se



ABSTRACT: The influence of solution pre-treatment on primary nucleation of *m*-hydroxybenzoic acid has been investigated through 550 cooling crystallization experiments. The metastable zone width has been determined at constant cooling rate and the time and temperature of the preceding superheating step have been varied. *m*-Hydroxybenzoic acid has two polymorphs and the influence of the polymorph used to prepare the solutions has also been investigated. There is an overall tendency in the experiments for the solution to exhibit a larger metastable zone width if it is superheated for a longer time and at a higher temperature, but under the investigated conditions this tendency is not very strong. The results show that the metastable form II preferentially crystallizes in all experiments and in particular when the solution has been more strongly superheated for several hours. However, when the time and/or the temperature of superheating is reduced there is an increasing tendency to obtain the stable form I. This is most clearly found for solutions prepared by dissolving form I. When the solutions are prepared by dissolution of form II, this tendency is weaker in what appears to be a systematic way. It is hypothesized that, unless the solution is strongly superheated for several hours, it will contain for a significant period of time clusters of solute molecules which can retain some degree of structure from the dissolved crystal. This leads to "memory" effects in the solution which may influence subsequent nucleation. The work includes a comprehensive review of previous published work on the influence of thermal history on nucleation in solutions and melts.

**Keywords**: thermal history, solution history, structural memory, nucleation, cluster, polymorphism.

## INTRODUCTION

The first experimental indications that the propensity for crystallization is sensitive to the preceding thermal treatment were discovered for crystallization from melts. In 1902, Schaum and Schoenbeck[1] reported that sufficient heating of the compound benzophenone above its melting point could inhibit nucleation during subsequent cooling. In 1907, Young and Burke[2] established qualitatively that both a prolonged time and an increased temperature of overheating led to a decreased tendency to crystallize during subsequent cooling, based on a large number of melt crystallizations of *p*-nitrotoluene. In work that followed, these findings were corroborated by observations reported for melts of organic compounds[3-5], metals[6] and polymers[7, 8].

Much later, similar effects were reported for crystallization of inorganic salts from aqueous solutions. The first attempt at a systematic study was presented in 1959 by Šmid et al.[9] for nucleation of inorganic salts, and in 1963 Nývlt[10] investigated the relationship between the time and temperature of overheating and the width of the metastable zone for nucleation of urea from aqueous solution. Nakai[11] performed crystallization experiments on aqueous solutions of sodium chlorate and based on kinetics of cluster distributions[12] developed a semi-empirical equation relating the temperature of undersaturation to the observed induction times. Further work on the effect of superheating on the metastable zone width[13-17] and induction time[17-19] has mainly served to establish qualitatively the effects previously reported. More recently, Burke et al.[20] found that increased superheating during crystallization of proteins resulted in a smaller number of nuclei, and Hussain et al.[21] found that the level of preceding superheating influenced the metastable zone width of vanillin and related organic compounds in various organic solvents.

The mechanisms possibly involved in the thermal history effects are still far from well understood or agreed upon. Proposals have been made along three conceptually different routes:

i) The first group of theories involve the concept of a structural memory within the solution itself. The assumption is that even in superheated solutions or melts, there will be a size distribution of molecular aggregates denoted clusters. The distribution is supposedly temperature-dependent, and the rate of transition upon change of temperature is sufficiently low to allow effects of previous thermal history to influence the cluster size distribution, and thereby the nucleation, during subsequent crystallization[10, 11, 13]. In the classical theory of nucleation, a cluster is a small aggregate of molecules with a crystalline structure, which grows or shrinks through the addition or removal of individual monomers. The distribution of clusters with different sizes $n_i$ is a function of temperature and supersaturation[22], through the free energy of formation of a cluster of size $i$:

$$n_i = n_1 exp\left[-\frac{\Delta G_i}{kT}\right] \quad (1)$$

where $n_1$ represents the total number of monomers in solution, and the free energy of formation of an $i$-cluster, $\Delta G_i$, depends on temperature and supersaturation[23]. Upon a sudden change in supersaturation, the time scale of readjustment of the cluster size distribution has been proposed to depend inversely on the diffusivity[24] or alternatively on the pre-exponential factor $J_0$ in the well-known classical nucleation rate equation[25]:

$$J = J_0 \exp\left[\frac{-\Delta G^*}{RT}\right] \quad (2)$$

Using the theoretical value[25] of $J_0$ results in very short redistribution times (fractions of a second)[16]. However, Nývlt has pointed out that the experimentally found values of $J_0$ are orders of magnitude lower than the theoretical value, and that the uncertainties inherent in the classical theory allows for a wide range of possible adjustment times. In the recently proposed two-step nucleation theory[26], it is postulated that the first step towards nucleation

involves solute molecules aggregating into a highly disordered phase, the physical properties of which would resemble those of a diffuse liquid droplet of high viscosity. The rate-limiting step is the restructuring within such dense disordered clusters into an ordered structure[27].
During the last decade, experimental evidence for the existence of clusters, both in supersaturated[28-31] and undersaturated solutions[32, 33], has been reported. Ginde and Myerson[34] analysed data from several studies and estimated that the average cluster size in metastable solutions is a function of its age and thermal history. For glutamic acid and L-histidine, the appearance of the conformational polymorphs is assumed to be related to clusters containing molecules of the corresponding conformation, and a possible interconversion between the two types of clusters is suggested[35].

ii) In the second theory, the effects of thermal history are explained by the survival of some crystalline particles during dissolution or melting for extended periods of time, being thermodynamically stabilized either by adhering to flat surfaces or more plausibly by residing in capillary cavities of the walls of the crystallizer or impurity particles[36]. These crystallites may then act either as seeds or as sources for secondary nucleation during subsequent cooling.

iii) In the third approach, it is assumed that heterogeneous solid particles present in the liquid, catalysing nucleation by lowering the energy barrier for primary nucleation, either dissolve/melt[22], or are somehow deactivated[4, 17, 37, 38], if subjected to increased temperatures for prolonged periods of time. Hypotheses based on this concept are strengthened by observations that careful purification of solutions by filtration reduces the propensity for nucleation[38], pointing to the importance of heterogeneous mechanisms in nucleation.

During the last 40 years the topic of history of solution has received little attention, and remains shrouded in mystery. Partly, the reason is probably that repeated primary nucleation experiments under identical conditions can exhibit large variations in the onset of nucleation[39] as well as in the distribution of appearance of different polymorphs[40], making it experimentally challenging to prove thermal history effects with sufficient statistical confidence. The aim of this contribution is to provide fresh experimental results on the influence of solution history, making advances along two different directions. The first is to build statistical confidence into the results by repeating each experiment a significant number of times. The second is to record the particular structure nucleating in each experiment. To our knowledge this is the first time that polymorphic outcome is analysed as a function of the history of a solution. The aim includes examination of the results against proposed theories. It is our hope that this work will initiate a renewed interest into the influence of the thermal and structural history of solution on nucleation, and our belief that further understanding can shed some light on clustering in solution and the process of nucleation itself.

*m*-Hydroxybenzoic acid, *m*HBA, is used as model compound, as it has two polymorphs with crystal structures solved[41], differing only slightly in solubility[42]. The thermodynamically stable polymorph at room temperature crystallizes in the monoclinic space group $P2_1/c$ and will henceforth be termed form I, whereas form II crystallizes in the orthorhombic space group $Pna2_1$. Forms I and II are monotropically related with a ratio of solubilities, and solid state activities, of approximately 1.3 at ambient conditions[42, 43]. The corresponding driving force for transformation from form II to form I is fairly constant between 10 and 50 °C, viz. 0.57–0.61 kJ/mol. On-going as well as previously reported work[42] has shown that *m*HBA exhibits selective crystallization of either form I or form II depending on the solvent, and at properly tuned conditions concomitant crystallization is rare. In the present work, experiments have been carried out on solutions prepared by dissolving either form I or form II, which have been kept superheated at different temperatures for different lengths of time. Solutions have been cooled at a constant cooling rate until nucleation, and the nucleation temperature and the nucleating polymorph determined. Each experiment has been repeated between 48 and 143 times.

## EXPERIMENTAL WORK

*Materials*

*m*-Hydroxybenzoic acid (CAS reg. no. 99-06-9, purity > 99%), shown in Figure 1, was purchased from Sigma-Aldrich and used without further purification steps. Ethyl acetate (Hipersolv, purity > 99.8%) was purchased from VWR.

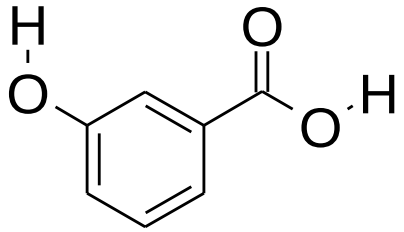


**Figure 1.** The molecular structure of *m*-hydroxybenzoic acid.

*Identification, preparation and solubility of the polymorphs*

Fourier-transform infrared (FTIR) spectroscopy has been used for polymorph identification on all isolated solid samples. A Perkin Elmer Spectrum One in attenuated total reflectance (ATR) mode, equipped with a ZnSe-crystal window, was used, with a scanning range of 650–4000 $cm^{-1}$ and a resolution of 4 $cm^{-1}$. The resulting FTIR spectra of the two pure polymorphs, shown in Figure 2, are easy to distinguish from one another. The purchased material was found to be consistent with form I, and was used as obtained without further purification. The same batch of purchased *m*HBA was used in all experimental work. The metastable form II was prepared by dissolving form I in pure ethyl acetate at 60 °C. The heated solution was filtered through PTFE membranes (pore size 0.2 µm) over to a new flask and moved to a cooling bath at around 10 °C. Nucleation of form II normally took place within a few minutes. The crystallized suspension was subsequently filtered and dried, and the crystals confirmed by FTIR to be form II.

The solubility of the two polymorphs in ethyl acetate at different temperatures was reported in a previous contribution[42]. The mole fraction solubility of the metastable form II is approximately 30% higher than that of form I at 10 °C and 24% higher at 50 °C.

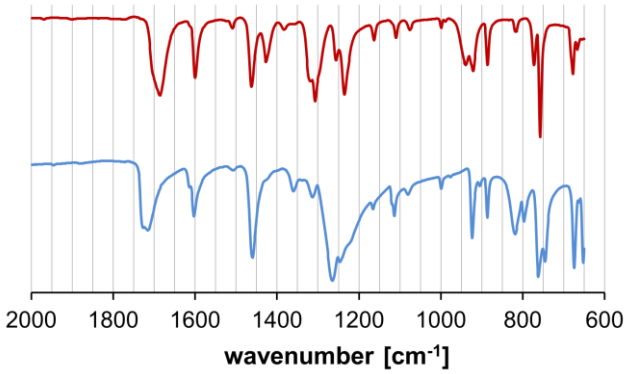


**Figure 2.** FTIR spectra of form I (red, upper) and form II (blue, lower) of *m*HBA.

*Cooling crystallizations*

Altogether 550 cooling crystallizations of *m*HBA in ethyl acetate solution have been performed, using a cooling rate of 5 °C/h. Solvent and cooling rate were chosen based on preliminary screening. It was found that primary nucleation was generally not concomitant; in all but one experiment, either the stable or the metastable polymorph was obtained in what appears to be pure form.

The following stepwise protocol, shown schematically in Figure 3, was employed in the creation and pre-treatment of solutions and ensuing crystallization experiments:

**Step A**: Crystals of *m*HBA form I were added, in an amount well in excess of solubility at 45 °C, to 250 ml ethyl acetate in a sealed flask, agitated using a magnetic stirrer bar at 400 rpm, placed in a thermostatic bath at 45 °C, and kept overnight.

**Step B**: The saturated solution, $C_s$ = 120.5 mg/g, was subsequently filtered through PTFE membranes (pore size 0.2 µm) into a clean bottle and kept at 60 °C for a period of at least 12 h. This procedure was carried out to ensure that any effect of solution memory at this stage would be negligible.

**Step C**: The solution temperature was then brought down to 50 °C, and an amount of either form I or form II (details in Table 1) was added to the solution, to reach a concentration corresponding to saturation at 50.0 °C with respect to the stable form I, $C_s$ = 132.0 mg/g; the corresponding saturation temperature of form II is 37.5 °C. Within a few minutes the suspension turned into a clear, transparent solution without visible crystals, also when form I was dissolved. The solution was left for 30 min at 50 °C.

**Step D**: The solution was then transferred (without filtration) into 20 ml test tubes, each tube being filled with ~15 ml of solution using pre-heated syringes. A magnetic stirrer bar was added to each test tube, which was then promptly sealed to prevent solvent evaporation and contamination. The test tubes were then moved directly to a second thermostatic bath at a temperature corresponding to a slight undersaturation with respect to form I (52, 55 or 60 °C) for a controlled period of time (8, 30 or >360 min). The temperature levels and time limits are marked with horizontal and vertical lines, respectively, in Figure 3.

**Step E**: The test tubes were then moved to a cryostatic bath (Julabo FP50) kept at 50 °C, and left under agitation for precisely 15 min to allow the solution temperature to reach 50 °C.

**Step F**: The test tubes were then cooled at a constant cooling rate of 5 °C/h until the solutions in all tubes had crystallized (step F). Temperatures corresponding to visible onset of nucleation were recorded with a camcorder, to within an estimated accuracy of ±0.1 °C. Nucleation was visibly observed as a rapid transformation from a transparent solution to a white slurry. As soon as sufficient crystal material was present in a test tube, the suspension was filtered and the isolated solids subjected to polymorph identification with FTIR.

For each experimental series, the described procedure was repeated for several batches of test tubes. The reason for using three steps (A, B and C) for the preparation of solutions was to limit the amount of the metastable form II required in order to make up the solutions in series 2 and series 6–9.

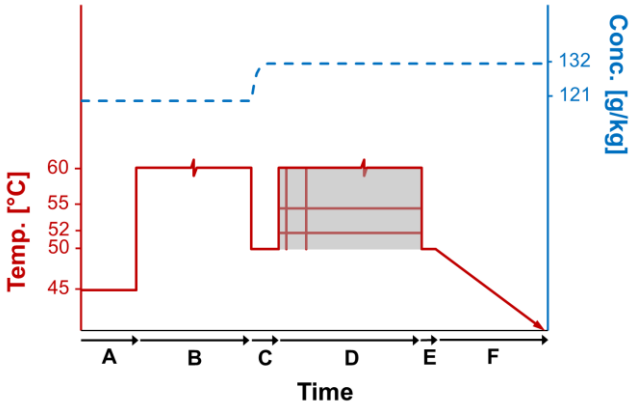


**Figure 3.** Schematic diagram of the protocol for pre-treatment and crystallization, showing the changes in temperature (red solid line) and solution concentration (blue dashed line) during steps A through F. The grey area represents conditions differing between the experimental series.

RESULTS

Nine series of experiments have been performed, with each series consisting of at least 47 repeatability experiments as specified in Table 1. In the first two series, differing only with respect to the dissolved polymorph, the temperature of superheating ($\Delta T_{SH}$) was high (10 °C) and the time ($t_{SH}$) very long (>360 min). The purpose of these two series was to establish a reference where any impact of solution history would be negligible or at least standardized. In series 3–5, the polymorph dissolved to create the solution was form I, and in series 6–9, form II was used. In all these experiments the superheating time was comparatively short, allowing the dissolved crystal material to influence the subsequent cooling crystallization. In Table 1, the first five columns represent the experimental conditions of each series. The remainder of Table 1 shows the number and fraction of experiments at each set of conditions that led to the respective polymorph crystallizing.

Table 2 gives the metastable zone width values, in the form of average temperatures of nucleation, for all experiments at each set of conditions producing form I, and the corresponding values for all experiments producing form II. The average driving force at the temperature of visible onset of nucleation, expressed as a difference in the chemical potential of the solute at the point of nucleation and at equilibrium ($\Delta\mu$), is listed for the two polymorphs. This value has been calculated for each respective polymorph using eq 3, where the activity ratio is approximated by the supersaturation ratio ($S$) using solubility data reported in Nordström and Rasmuson (2006)[42]:

$$\Delta\mu = RT \ln\left(\frac{a}{a_{eq}}\right) \cong RT \ln\left(\frac{x}{x_{eq}}\right) = RT \ln S \qquad (3)$$

where $R$ is the gas constant, $T$ is the temperature and $x$ is the solute mole fraction.

**Table 1.** The nine series of nucleation experiments and the polymorphic outcome.

| Series | Polymorph | $\Delta T_{SH}$ [a] | $t_{SH}$ [b] | No of | Crystallizing polymorph | | | |
|---|---|---|---|---|---|---|---|---|
| no | | [°C] | [min] | exp | *Form I* | | *Form II* | |
| | | | | | No | Fraction | No | Fraction |
| 1 | Form I | 10 | >360 | 143 | 14 | 9.8% | 129 | 90.2% |
| 2 | Form II | 10 | >360 | 59 | 4 | 6.8% | 55 | 93.2% |
| 3 | Form I | 10 | 30 | 48 | 19 | 39.6% | 29 | 60.4% |
| 4 | Form I | 5 | 30 | 48 | 18 | 37.5% | 30 | 62.5% |
| 5 | Form I | 2 | 30 | 48 | 24 | 50.0% | 24 | 50.0% |
| 6 | Form II | 10 | 30 | 48 | 7 | 14.6% | 41 | 85.4% |
| 7 | Form II | 5 | 30 | 47 | 10 | 21.3% | 37 | 78.7% |
| 8 | Form II | 2 | 30 | 64 | 23 | 35.9% | 41 | 64.1% |
| 9 | Form II | 2 | 8 | 48 | 7 | 14.6% | 41 | 85.4% |

a) The superheating temperature during step D with respect to the temperature of saturation of form I, 50 °C.
b) The time of the superheating step D.

**Table 2.** Average nucleation temperatures for all nucleations and for each polymorph, and the average thermodynamic driving force at nucleation, with 95% confidence limits, by experimental series.

| Series | Avg $T_{nucl}$ [°C] | | | Avg $\Delta\mu$ at $T_{nucl}$ [kJmol$^{-1}$] | |
|---|---|---|---|---|---|
| no | *All cases* | *Form I* | *Form II* | *Form I* | *Form II* |
| 1 | 17.51 ± 0.60 | 20.85 ± 3.28 | 17.15 ± 0.55 | 1.20 ± 0.12 | 0.75 ± 0.02 |
| 2 | 17.35 ± 0.71 | 19.78 ± 7.83 | 17.10 ± 0.67 | 1.24 ± 0.29 | 0.74 ± 0.02 |
| 3 | 20.72 ± 0.60 | 21.79 ± 0.95 | 20.02 ± 0.73 | 1.16 ± 0.04 | 0.65 ± 0.02 |
| 4 | 19.36 ± 0.95 | 21.03 ± 1.98 | 18.36 ± 0.93 | 1.19 ± 0.07 | 0.71 ± 0.03 |
| 5 | 21.38 ± 0.96 | 23.16 ± 1.46 | 19.61 ± 0.95 | 1.11 ± 0.05 | 0.66 ± 0.03 |
| 6 | 19.19 ± 0.56 | 21.61 ± 2.33 | 18.77 ± 0.48 | 1.17 ± 0.09 | 0.69 ± 0.02 |
| 7 | 20.10 ± 0.63 | 21.58 ± 2.31 | 19.70 ± 0.54 | 1.17 ± 0.09 | 0.66 ± 0.02 |
| 8 | 20.37 ± 0.97 | 22.66 ± 1.76 | 19.09 ± 1.01 | 1.13 ± 0.07 | 0.68 ± 0.03 |
| 9 | 20.64 ± 0.89 | 25.52 ± 1.78 | 19.80 ± 0.76 | 1.02 ± 0.07 | 0.66 ± 0.03 |

*The stochastic nature of nucleation*

In the present work, in order to properly account for the random nature of the process in evaluating the effect of solution history on nucleation, all experiments were repeated between 47 and 143 times. 95% confidence limits were established and differences and trends in the average values were analysed across different experimental series. Admittedly, a higher number of experiments in all cases would have been desirable, but since the nucleating polymorph had to be established off-line by FTIR spectroscopy the amount of work required became limiting. The results reveal a considerable variation in the onset of nucleation between individual test tubes under identical experimental conditions. For the reference series, the highest and lowest recorded nucleation temperatures for form I nucleations were 29.4 °C and 8.6 °C respectively, corresponding to a difference in time of over 4 h, and a driving force ranging between 0.87–1.63 kJ/mol, i.e. a difference of 0.76 kJ/mol. For form II nucleations, the maximum and minimum nucleation temperatures were 22.8 °C and 6.5 °C, respectively, corresponding to a time difference of 3.5 h, and a driving force range of 0.55–1.07 kJ/mol, i.e. a difference of 0.52 kJ/mol. Furthermore, in all of the series of cooling crystallizations, both known polymorphs were obtained, but the crystals in each individual tube were isolated as polymorphically pure. These results point to the stochastic nature of nucleation, and stress the fact that reliable information on nucleation kinetics, especially for a polymorphic compound, can only be obtained from a sufficiently large data set[40].

*Influence of solution pre-treatment on the onset of nucleation*

The results reveal an influence of the thermal history on the nucleation temperature, manifested as a reduction of the MZW when the superheating step is shortened. In these series, the solutions were kept undersaturated for more than 6 h at 60 °C, i.e. 10 °C superheating with respect to form I, and solutions were prepared by dissolving either form I (series 1) or form II (series 2). As shown in Table 2 and Figure 4, the difference in nucleation temperature between the two reference series is very small, and well within the statistical error margin. Across all experimental series, the lowest average nucleation temperatures were obtained in series 1 and 2. Overall, the difference in average nucleation temperature between series 1–2 on the one hand and series 3–9 on the other is 2.2 °C. This difference in nucleation temperature is observed for both polymorphs, as shown in Figure 4. When the time of superheating was reduced to 30 min, the metastable zone width was reduced by 1.5 °C on average for nucleation of form I and 2.1 °C for nucleation of form II, as compared to the reference series. This corresponds to decreases in the average driving force at nucleation of 0.06 kJ/mol (5%) and 0.07 kJ/mol (9%), respectively. The effect is more apparent for those cases where form II nucleated. Because of the comparatively few nucleations of form I in series 1 and 2, the effect is not statistically established at the 95% confidence level for this polymorph.

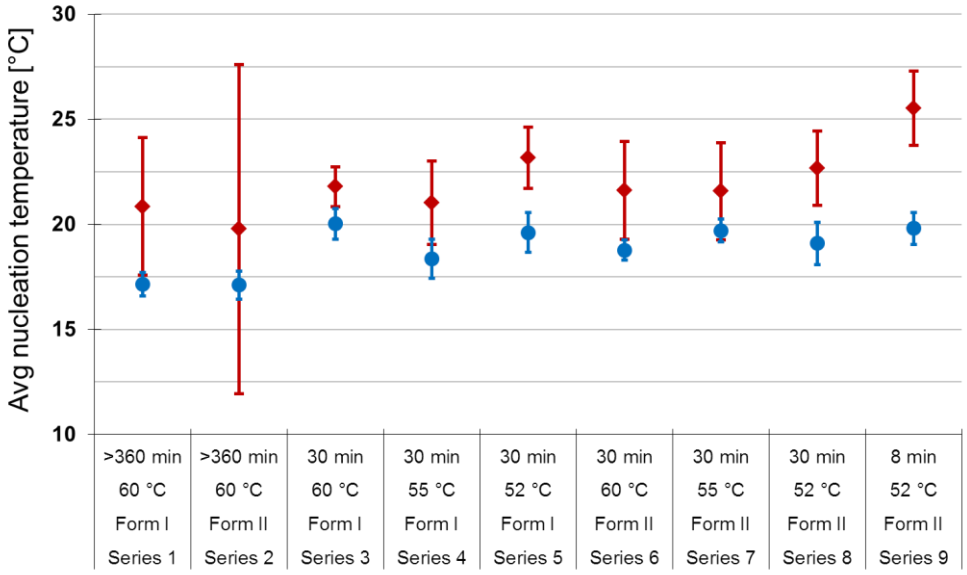


**Figure 4.** Average onset temperature of nucleations of form I (red diamonds) and form II (blue circles), with 95% confidence limits, for the 9 experimental series.

*Influence of solution pre-treatment on the nucleating polymorph*

The results on the influence of solution pre-treatment on the crystal structure of the solid that nucleates upon cooling are summarized in Figure 5. As shown in the figure, the difference in average fraction of nucleating polymorph between the two reference series 1 and 2 is small. Statistically no difference can be established, using 95% confidence limits calculated by the Wilson equation[44] without correction for continuity. Since the same applies to the average nucleation temperatures of both polymorphs, it appears that by employing a superheating temperature of 10 °C for 6 hours, a reference state is created where at least the effect of the dissolved polymorph has disappeared. Under the experimental conditions employed, *m*HBA shows a distinct preference to nucleate as form II, despite the fact that the supersaturation is higher for form I.

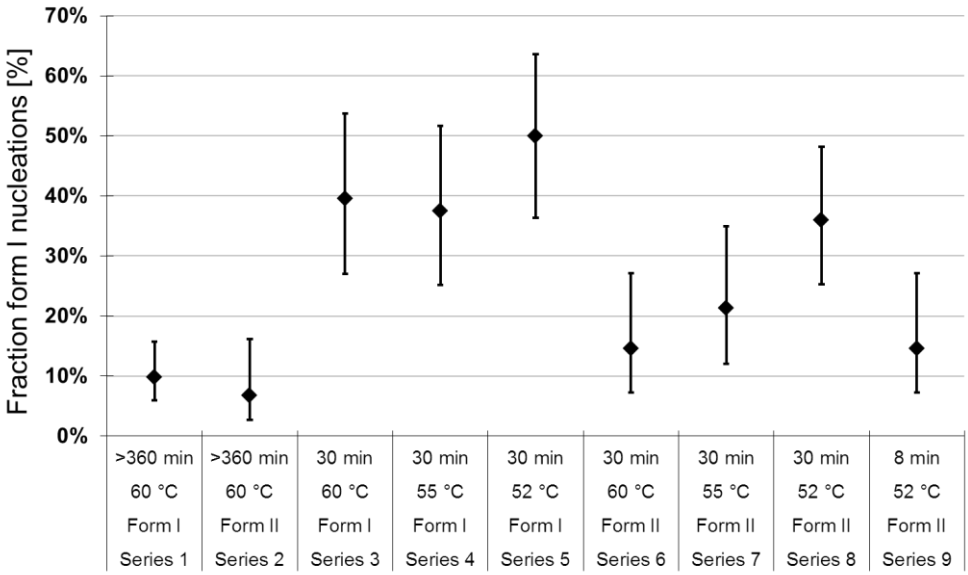


**Figure 5.** Fraction of form I nucleations, with 95% confidence limits, for the 9 experimental series.

The experimental results show that:

i) solutions prepared by dissolving form I which are superheated by between 2 and 10 °C for 30 min (series 3–5) resulted in a substantial increase in the fraction of form I nucleations compared to the reference series 1–2. For the case $\Delta T_{SH}$ = 10 °C, the fraction of form I nucleations is more than four times higher than in the reference case, and this difference is statistically significant at the 95% level.

ii) solutions prepared by dissolving form II which are kept superheated at $\Delta T_{SH}$ = 2 °C for only 8 min (series 9) resulted in an approximately similar high fraction of form II nucleations as the reference series 1–2. However, when the superheating time was extended to 30 minutes (series 8) the fraction of form I nucleations increased almost to the level obtained when form I was dissolved.

iii) for solutions prepared by dissolving form II, when the superheating time is kept constant at 30 min, the average fraction of form II nucleations shows a tendency to decrease with reduced superheating temperature (series 6–8). For the case $\Delta T_{SH}$ = 10 °C, the outcome is not far from that of the reference series, but with decreasing $\Delta T_{SH}$ the fraction of experiments resulting in form I increases. Data for solutions prepared using form I (series 3–5) indicate a similar trend i.e. that nucleation of form I is promoted by a lower superheating temperature.

iv) at equal superheating temperatures and superheating time (30 min), the polymorphic outcome is shifted systematically towards an increased fraction of the polymorph that was dissolved, as given by comparing the results of series 3 with series 6, 4 with 7, and 5 with 8.

## DISCUSSION

The work has shown that nucleation of *m*HBA from an ethyl acetate solution saturated at 50 °C depends both on the thermal and the structural history of the solution. Using a fairly moderate superheating temperature for a limited time results in a narrower metastable zone for nucleation of either polymorph, and affects the polymorphic outcome. The complexity of the observed results suggests that an explanation based on the melting or deactivation of colloidal heteroparticles is insufficient. Regarding theories based on undissolved crystal particles, although there should be no doubt that all solid particles in the solution bulk are fully dissolved before cooling starts, there is a possibility of extended survival of microcrystals by adhering to capillary pores. Such a mechanism could explain some of the results. However, in order to reconcile this explanation with the results of series 6–8, which indicate that nucleation of form I is promoted by the dissolution of form II, complementary mechanisms would be required. In order to shed some lights on this, complementary experiments were performed. Solutions were prepared with the same concentration as in series 1–9, i.e. 132.0 mg/g. The solutions were kept at superheated conditions ($\Delta T_{SH}$ = 10 °C) for more than 6 h and then cooled to 40 °C, at which point the solutions were supersaturated with respect to form I but undersaturated with respect to form II. Seeds of the form II material were then added to the solution. No crystallization was observed after 2 days at 40 °C in four repeated experiments. When solutions being treated the same way were seeded with form I material, crystallization of form I took place immediately. The results of these experiments exclude the possibility of microcrystals of form I existing in the form II seeds, and indicate that polymorph transformation from form II into form I, either in the solution bulk during dissolution step or for crystals surviving in capillary pores, is very unlikely.

It appears that the most plausible explanation for the memory effects observed in this work involve structuring of solute molecules in solution. In a solution with a reasonably high concentration there will be clustering of solute molecules, and if given sufficient time, the cluster size distribution will reach a dynamic steady-state which depends on temperature and concentration[23, 34]. Little is known about the exact properties of these clusters but it may be hypothesized that there are also distributions in terms of cluster shape and structure. Upon changes in temperature or concentration the solution will gradually adjust into a new steady-state situation, and critical to our interpretation of the experimental results is

that this process is assumed to require minutes or hours, rather than milliseconds or seconds. We are not aware of any data which clearly reveal the time constants for cluster redistribution. However, both dominant nucleation theories allow for quite long cluster redistribution times[16, 26]. In addition, mechanisms can be envisaged by which redistribution times can be prolonged by the presence of heteroparticles or imperfections in the vessel walls, giving sustained survival to transient structures. Against this background, it is our hypothesis that the dissolution of a crystalline solid may temporarily lead to a clustering situation in the solution deviating from the steady-state. In time the solution will gradually transform to correspond to the steady-state given by the prevailing temperature and concentration, but until then the presence of molecular clusters structurally reminiscent of a dissolved polymorph could temporarily shift the balance in favour of re-nucleation of that particular polymorph. As shown in Figure 5, a comparison of the results of series 1 and series 3 suggests that shortly after dissolution of form I the superheated solution will contain solute molecular arrangements which promote nucleation of form I, but if sufficient time is given at a superheating of 10 °C, these arrangements will gradually transform and the state of the solution will approach a steady-state promoting nucleation of form II.

However, the experimental results show a more complex behaviour than this. As given by comparing the results of series 2, 8 and 9, when form II is dissolved and the solution is mildly superheated ($\Delta T_{SH}$ = 2 °C), nucleation of form II is promoted if the time of superheating is short, but nucleation of form I is promoted when the superheating time becomes longer. Accordingly, further to our hypothesis, the transient molecular clusters emanating from the dissolution process could be characterized by two components: structure and solute concentration. When the structural features promoting nucleation of form II are lost, remaining is still a promoted nucleation overall, and in particular of form I. The results suggest that in a superheated solution, any structural memory of the dissolved crystal will be lost before the concentration component. This concept is illustrated in Figure 6.

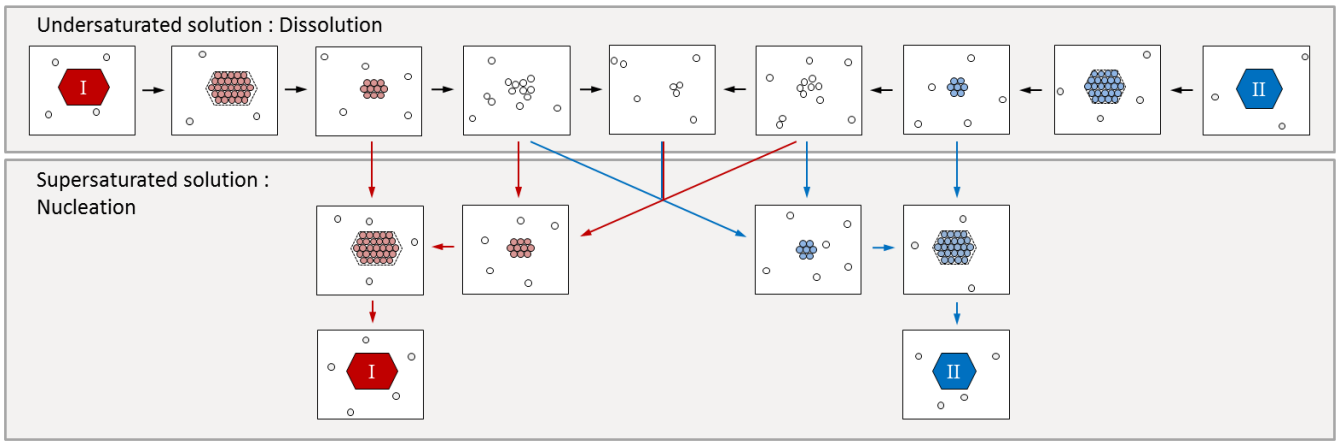


**Figure 6.** Possible pathways of re-nucleation in a solution starting with dissolution of form I and form II, respectively.

Comparing the results of experiments carried out at different $\Delta T_{SH}$, there is a trend of increasing fraction of form I nucleations observed in series 6–8 with decreasing superheating temperature, and the data from series 3–5 are at least not contradicting this trend. It is not possible to say whether this emanates from a steady-state cluster distribution which is appreciably different at 60 °C compared to that at lower temperatures, or simply from a slow transformation from the dissolution conditions. Nucleation temperatures are much lower than the pre-treatment temperatures, and the rate of cooling is relatively low, so it is surprising that the superheating temperature does play such a strong role. On the other hand, the data overall suggest that transformation rates are low. For example, the polymorphic outcome of series 1 and 3, which differ only with respect to the duration of the superheating step, suggest that the memory of the dissolution survives not only the time of undersaturation during step D (and for dissolution of form II also most of step C), but also the 6–8 hours of cooling before nucleation

occurs. One factor in this is of course that a reduced temperature in general slows down kinetic processes.

The difference in solubility will lead to a difference in the dissolution process for forms I and II. In the experiments prepared from form I, the dissolution chemical potential difference, calculated using eq 3, is initially equal to -0.23 kJ/mol, decaying to zero at the end of the dissolution process. In the case of dissolving form II, the initial dissolution driving force is equal to -0.80 kJ/mol, decreasing to -0.57 kJ/mol at completion of dissolution. During the entire dissolution process, the solution will be well undersaturated with respect to form II, whereas the solution towards the end of step C is actually saturated with respect to form I. Consequently, form II will dissolve more rapidly, and the dissolved state will be reached earlier, leaving more time (up to 30 minutes) for molecular rearrangement in solution. This could explain the fact that the structural memory seems to last longer for dissolution of form I compared to form II.

In Figure 7, the fraction of form I nucleations in excess of the corresponding value for the reference series is plotted against the driving force for dissolution, for series 3–8. Specifically, the x-axis coordinate is the solute chemical potential difference between the solution at complete dissolution in step D, and the saturated solution of the polymorph being dissolved, and the y-axis coordinate is the fraction of form I nucleations divided by the same number for the reference series. Assuming that $-RT \ln S_A$ can be taken as a measure of the driving force for deletion of the solution structural history of a dissolved polymorph A, one interpretation of Figure 7 is that the structural state of the solution approaches the reference state more quickly with increasing degree of undersaturation during step D.

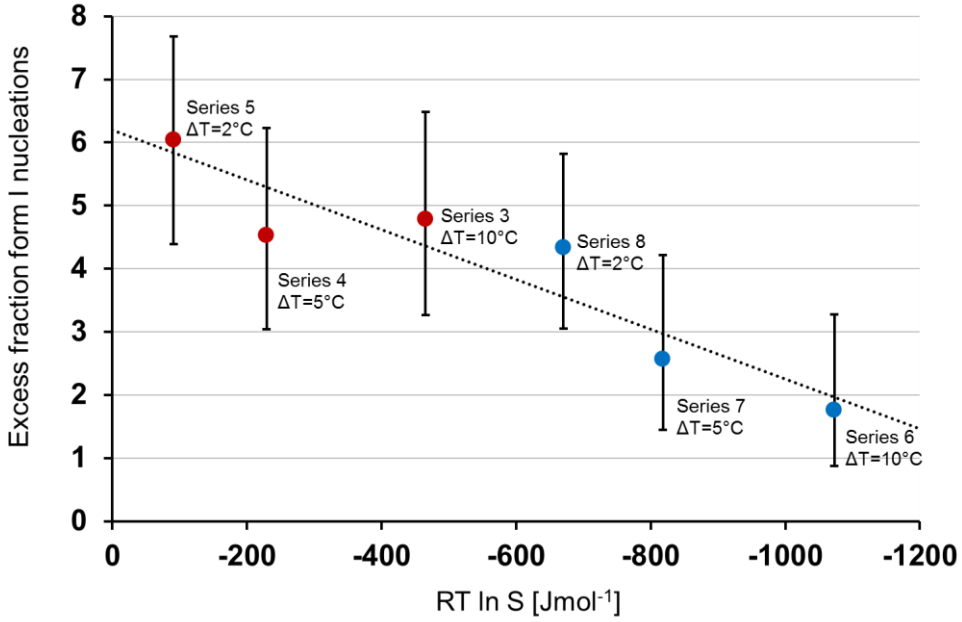


**Figure 7.** Excess fraction of form I nucleations relative to the reference series plotted against the undersaturation during step D, expressed as $RT \ln S$, for series 3–5 (red) and 6–8 (blue). The data are correlated with a dotted line ($R^2 = 0.87$).

Another aspect is that the maximum concentration in the boundary layer surrounding a dissolving form II crystal is 167 mg/g – the solubility of form II at 50 °C – while the corresponding solubility of form I is only 132 mg/g. This means that the solution close the surface of dissolving form II crystals will be supersaturated with respect to form I by 0.57 kJ/mol. In theory, this could lead to form I nucleating during dissolution of form II. As given by reference series 1, form I nucleates on average at a temperature of 20.6 °C, corresponding to a driving force of 1.21 kJ/mol, i.e. clearly higher than the supersaturation in the boundary layer of dissolving form II crystals. However, since the temperature is 30 °C higher than this value during the dissolution step the possibility of primary 3D nucleation of form

I in the boundary layer of the dissolving form II crystals cannot be ruled out. In addition, there are examples in the literature[45, 46] and in unpublished on-going work of the possibility of facilitated nucleation of the stable polymorph on the surface of crystals of a metastable form. A comparison of the polymorphic outcome of series 8 and 9 suggests, however, that a net promotion of form I nucleation only develops after some time, which does not really fit into a mechanism of nucleation of form I in the boundary layer or on the surface of dissolving form II crystals.

To summarize, at the present stage of development where i) a firm theory of crystal nucleation itself is still lacking and ii) there is an insufficient understanding of the structuring of organic molecules in solution, we cannot provide a comprehensive and conclusive explanation of all observable effects of varying the history of a solution. Through the present contribution, however, statistically verified experimental evidence in support of the existence of solution history effects is provided, and polymorphic outcome is added to the portfolio of experimentally observable effects. The significant increase in the fraction of form I nucleations, in particular in series 3–5 and series 8 as compared to the reference series 1–2, shows that the immediate thermal history of a solution can have an impact on the nucleation temperature and the nucleating polymorph. A possible explanation is that the dissolution process leaves partially structured molecular assemblies of higher solute concentration in the solution, which will gradually rearrange themselves in accordance with the prevailing temperature and solute concentration in the solution. However, this rearrangement process takes time and the rate is dependent on the temperature. In a cooling crystallization following immediately upon complete dissolution, re-nucleation of the polymorph that was dissolved is promoted, while after some further time nucleation of form I is promoted regardless of the dissolved polymorph.

## CONCLUSIONS

The present contribution provides statistically verified experimental evidence in support of the existence of solution history effects, not only observed as facilitated nucleation, but also observed as a change in the polymorphic outcome. The results show that nucleation of *m*-hydroxybenzoic acid in ethyl acetate upon cooling depends on the polymorph being dissolved to create the solution as well as on the superheating time and temperature. A milder superheating of the solution in terms of time and temperature prior to cooling results in a narrower metastable zone width for both polymorphs, and alters the polymorphic preference of the nucleation process. In the reference case, the solution was superheated for 6 hours at 10 °C ($\Delta T_{SH}$ = 10 °C) above the saturation temperature of the stable form I. When the time of superheating was reduced to 30 min, the metastable zone width was reduced by 1.5 °C for nucleation of the stable form I and by 2.1 °C for nucleation of the metastable form II.

For solutions pre-treated by superheating at $\Delta T_{SH}$ = 10 °C for more than 6 h no influence of the dissolved polymorph on nucleation was observed, and in about 90% of the experiments form II nucleated. However, when superheating by between 2–10 °C for 30 min, solutions prepared by dissolving form I resulted in a four- to fivefold increase in the fraction of form I nucleations compared to the reference case. For solutions prepared by dissolving form II and superheating by 2 °C for 8 minutes, the resulting polymorphic fractions were the same as in the reference case, whereas superheating by 2 °C for 30 minutes surprisingly resulted in a higher fraction of form I nucleations than the reference case.

The results of the present work exhibit a substantial variation both in nucleation temperature and polymorphic outcome for experiments performed under identical conditions. However, by performing a significant number of equal experiments, reasonable statistical confidence can be obtained.

NOTATION

| | | |
|---|---|---|
| $a$ | Activity | [mol/mol] |
| $C_S$ | Saturation concentration | [mg / g solvent] |
| $\Delta G_i$ | Gibbs free energy of formation of i-cluster | [J] |
| $\Delta G^*$ | Gibbs free energy of formation of critical cluster | [J] |
| $J$ | Rate of nucleation | [$s^{-1}$ $m^{-3}$] |
| $J_0$ | Pre-exponential factor in nucleation rate equation | [$s^{-1}$ $m^{-3}$] |
| $k$ | Boltzmann constant | [J $K^{-1}$] |
| $n_i$ | Number of *i*-clusters | |
| $R$ | Gas constant | [J $mol^{-1}$ $K^{-1}$] |
| $S$ | Supersaturation ratio | |
| $T$ | Temperature | [K] or [°C] |
| $\Delta T_{SH}$ | Superheating temperature | [K] or [°C] |
| $t$ | Time | [min] |
| $t_{SH}$ | Time of superheating | [min] |
| $x$ | Mole fraction | |
| $\Delta\mu$ | Difference in solute chemical potential | [J $mol^{-1}$] |

*Subscripts*

| | |
|---|---|
| eq | At equilibrium |
| nucl | At nucleation |

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