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Seeding the Desired Polymorph: Background, Possibilities, Limitations, and Case Studies

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Abstract:

The techniques for seeding of a desired polymorph during crystallisation from solutions are reviewed. The basic information about the type of polymorphism needed to consider seeding strategies is discussed first. The development of seeding strategies is facilitated by certain data on the system. Their relevance as well as techniques for the determination are given. Reflections on the choice, characterisation, and preparation of the seed are followed by a discussion of techniques for the determination and optimisation of the seeding, that is, the rate of crystallisation and amount of seed added. Scale-up issues from the well-controlled environment of the laboratory to the plant sum up this point. The discussion of published as well as unpublished results of seeding strategies serve as illustration of the theoretical and practical considerations made.

Introduction

Polymorphism is a keyword of considerable importance in the life-sciences and especially in the pharmaceutical industry. It abridges the fact that a solid compound can exist in different crystalline forms that can have different physical chemical properties. To ensure no variations in the product to be due to different solid-state properties, care must be taken in selecting the most appropriate solid state form for the substance and in ensuring a reproducible production of this form.

As concerns the pharmaceutical industry, it has been shown that more than half of the drug substances described in monographs crystallise in more than one solid state form, being it either polymorphs, solvates, or both.[1] The solid state form of a drug substance can influence a variety of properties, namely the solubility and rate of dissolution or the chemical stability or stability against excipients. Thus, the regulatory bodies require an exhaustive search for polymorphic forms of a drug substance.[2,3] The manufacturer is required to make a substantiated choice for one of the forms, or a defined mixture of forms. Changes in the polymorphic form of the batches produced are seen as indicative of changes in the production process, also requiring the reproducible crystallisation of a certain solid state form.

The choice of the solid-state form of a new drug substance is up to the applicant and should be made by considering all aspects, such as chemical stability and stability against excipients, dissolution behaviour and bioavailability, and last but not least thermodynamic stability of the solid state form and ease and reproducibility of production.

Crystallisations in the pharmaceutical industry are most often carried out batch-wise. The techniques used are cooling, evaporation, drowning-out, and reaction crystallisations. Normally, the crystallisation will be carried out as unseeded crystallisation, relying on spontaneous nucleation and the modification it entails. In a large number of cases, this will be an unstable modification, as is predicted by Ostwald's rule of stages.[4] However, an unstable form is prone to a phase transformation either while the product is still in suspension, during work-up, or even during storage, although the latter transformation is rarely observed.

Instead of relying on the modification formed under spontaneous nucleation, control can be exercised on the modification formed. One of the most straightforward techniques is the control of the nucleation process via seeding.[5]

Seeding in crystallisation can be likened to seeding in agriculture, a process that has inspired painters such as van Gogh to some superb work, like the "Sower with Setting Sun" to be found in the van Gogh Museum in Amsterdam. Seeding in agriculture is exercised during germination by planting seeds of the desired form—however, it is also exercised by ensuring the survival of the only type of crop desired, a technique that has yet to find its way into the crystallisation of drug substances.

Seeding techniques are mostly applied for batch crystallisations. In continuous crystallisers nucleation occurs mostly via secondary nucleation, where seeding is sometimes used during start-up and to dampen-out oscillations of the crystalliser. For batch-wise crystallisation in the chemical industry, four major applications can be identified:

to start the crystallisation, for example, if the system crystallises with difficulties or if it forms an oil,

to control the crystal size distribution, in most cases to produce large crystals with a narrow size distribution and to avoid fines (the most prominent example being sugar boiling to produce household quality, where *footing* ensures large grains having a narrow size distribution),

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(4) Ostwald, W. *Z. Phys. Chem.* **1897**, *22*, 289.
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to avoid encrustation through spontaneous nucleation, and the control of the polymorphic form, although this issue is rarely discussed in the literature.

Another important application of seeding is in single-crystal-growth, where seeding is necessary to obtain crystals of high purity and especially very high perfection and with the desired orientation.

This paper discusses seeding to control the polymorphic modification in batch-wise crystallisation processes. First, a review is given of the types of polymorphism encountered and the basic data that should have been gathered on the system. These data help in characterising the type of polymorphism of the system and to judge the possibilities as well as limitations to seeding the system. These discussions will be followed by a short review on the spontaneous—unseeded—formation of polymorphs and the possible fate of polymorphs from nucleation to the dry product. For the seeding process itself, it will be shown what can be done with seeding and what should be asked from seeding. Finally, the preparation of the seed and the operational details of the seed addition will be presented.

The discussions will be summed up by case studies.

Theoretical Background: Basic Data

Characterisation of the System. Prior to any attempt to control the modification via seeding, basic information on the systems must have been gathered, that is, different polymorphs of the substance must have been identified, techniques must exist to characterise the polymorphic form of the product, and the formation under different conditions should have been elucidated. In the following, important information on the system and their relevance as well as techniques for their determination will be discussed.

1. Solubility. Important data on the system can be derived from its phase diagram, containing information on the solubility as well as stability of the different phase. It is not necessary to determine the full phase diagram, in most cases, determining the solubility as a function of temperature suffices.

Before determining solubility, an unambiguous choice for the units of solubility must be made. Throughout this text, the units used are mass of solute on a solvent-free basis either per volume or mass of solvent on a solute-free basis. This choice has proven very practical in the work.

For solubility determination, the flask method normally suffices. Solute and solvent are placed in sealed containers, for example, vials, and stored at constant temperature for a couple of days. Samples of the solution are withdrawn, filtered if necessary, and analysed for content of solute. The residue is checked for the polymorphic form. This will lead to the solubility as a function of temperature and modification of the residue. Figure 1 gives the experimental results for the solubility of the anhydrate and hydrate form of a β-carboline, where the solid state from changes as a function of the water content of the solvent.[6] Due to the ready transformation of the solute into the stable from, only the solubility of the stable modification could be determined. Thus, only the form stable or kinetically sufficiently stable at a certain temperature can be accessed by this technique. Still, the data show the hydrate to be stable at a water content of 4% at 0 °C and 8% at 25 °C.

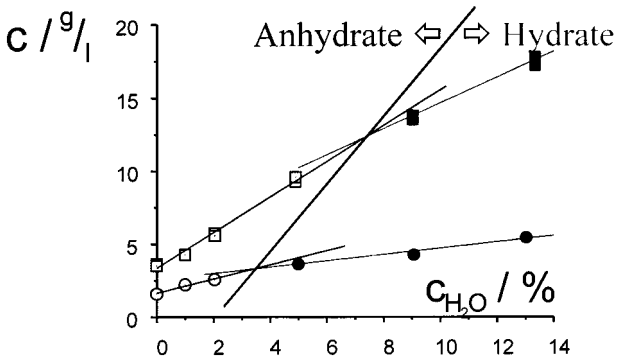

Figure 1. **Solubility of pyrazzocarnil hydrochloride in ethanol—water mixtures at 0 °C, circles and at 25 °C, squares. With increasing water concentration, the form of the residue as determined by XRPD changes form anhydrate I, open symbols, to hydrate I, full symbols. Also indicated is the border between anhydrate and hydrate as a function of temperature.**

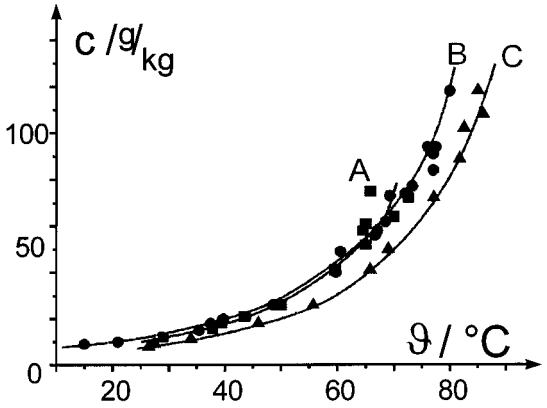

Figure 2. **Solubility of the A, B, and C modification of abecarnil in isopropyl acetate as determined by the bracketing technique.[8] Note the solubility of all modifications could be determined well into the region of instability.**

A more elaborate, but rather tedious technique to determine the solubility of stable as well as unstable modifications is the bracketing technique.[7,8] This technique uses synthetic solutions and observing the growth and dissolution of crystals of the modification under investigation under a microscope. Figure 2 shows results for the solubility of the A, B, and C modification of abecarnil, another β-carboline in isopropyl acetate. Note, that the solubility of the unstable A and B modification could be determined in the entire range. This is mainly due to the fact, that seeds of a more stable form can be excluded efficiently via this technique.

The data show the C modification to be the stable form at all temperatures, having the lowest solubility. The A and B modification form an enantiotropic pair with a cross over temperature of ~60—70 °C.

The relative stability of modifications can also be assessed by using a mixture of both—or all—forms to prepare a

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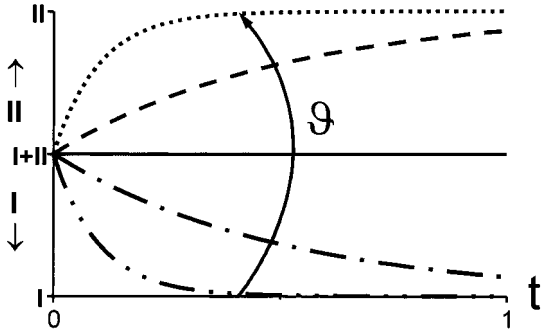

Figure 3. **Schematic rendering of the development of a mixture of two modification, I and II, during storage in suspension at five different temperatures. At low temperatures, the mixture will develop into form I, while with increasing temperature, form II will develop. The experiments give information on the temperature domains of the stability of the two forms.**

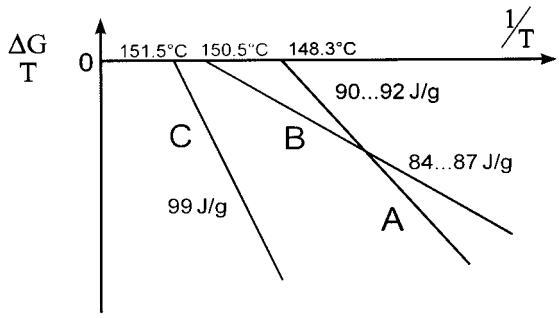

Figure 4. **Enthalpy−temperature diagram for abecarnil.[8] The substance forms three modifications, A, B, and C. Given are the temperatures and enthalpies of fusion as determined by DSC. The C modification is the stable form at all temperatures, that is, monotropic with respect to A and B. The A and B modification form an enantiomorphic pair with a cross over temperature of ~80 °C. The difference to the 60−70 °C estimated from solubility measurements is within the experimental error of both techniques.**

suspension. The mixture is suspended in a moderate solvent, and the development of the modification of the mixture is followed with time. Except for the case where the polymorphic form of the residue is unchanged, for example due to a very slow kinetics, the residue will transform into one of the forms, the stable one, independent of nucleation of the stable form,[9] Figure 3.

This approach also applies to solvates, provided that the solvent of solvatisation is present in the mother phase and its concentration stays constant. If these conditions favour a solvate, it will be formed from an ansolvate, or if two different solvates compete, the stable one will develop.

The measurements of solubility and especially the assessment of differences in solubility between different forms should be corroborated. An initial test of consistency can be made by plotting the data. More elaborate schemes involve independent measurement techniques.

For polymorphs, a plot of concentration versus temperature must yield the lowest solubility for the stable form. By plotting the data in a so-called van't Hoff plot, that is, by plotting the log of concentration expressed in molar fraction versus the inverse of the absolute temperature, ln x versus $1/T$, the enthalpies of dissolution of the forms can be calculated, which are related to thermoanalytical data as the heat of fusion:

$$\frac{\mathrm{d}\ln c}{\mathrm{d}\,1/T} = \frac{\Delta_\mathrm{d}H}{R} \approx \frac{\Delta_\mathrm{f}H}{R}$$

The heat of dissolution does not always equal the heat of fusion. However, the difference in solubility between two modifications always equals the difference in heats of fusion:

$$\frac{\mathrm{d}\ln c}{\mathrm{d}1/T}\Big|_\mathrm{I} - \frac{\mathrm{d}\ln c}{\mathrm{d}1/T}\Big|_\mathrm{II} = \frac{\Delta_\mathrm{tr}H}{R}$$

The use of thermoanalytical data in an enthalpy-temperature diagram will help in identifying the stable form and temperatures of transition.[10,11] For a polymorphic system the melt is an appropriate reference state. From this state, the free enthalpies ΔG of formation of the polymorphs can be written as $\Delta_\mathrm{f}G = \Delta_\mathrm{f}H - T\Delta_\mathrm{f}S$, with $\Delta_\mathrm{f}S = \Delta_\mathrm{f}H/T_\mathrm{f}$, where T_f is the temperature of fusion. Rearranging to $\Delta_\mathrm{f}G/T_\mathrm{f} = \Delta_\mathrm{f}H(1/T - 1/T_\mathrm{f})$ gives the free enthalpies as straight lines with $\Delta_\mathrm{f}G = 0$ at $T = T_\mathrm{f}$, Figure 4.

It is noted that the cross-over temperature between different forms is independent of the solvent as is the degree of difference between the forms. The form with the lowest free enthalpy is the stable form at the given temperature, no matter in which mother phase.

In the case that no polymorphic transition occurs in the solid state until the melting point, information on the relative stability of the polymorphs can also be derived from these temperatures. The modification with the highest melting point is the stable one at its melting temperature. Lower temperatures can be covered by mixing the crystals with compounds that easily form eutectic mixtures and thus lowering the melting point.[12]

These measurements and reflections on the thermodynamics can also help in extrapolating the solubility to temperatures, where the form is unstable, and in judging the degree of supersaturation of the metastable form, that is, its degree of metastability.

2. Metastability. The width of the metastable zone is of considerable importance in determining the possibilities and operation conditions for seeded crystallisations. Consider a solution initially undersaturated is cooled or concentrated by solvent evaporation, Figure 5. After the line of solubility is crossed, spontaneous nucleation will not occur immediately. Instead, the solution has to be cooled, or concentrated to a certain concentration−temperature point, until spontaneous nucleation occurs. This is the border of the metastable zone. This border is not a clearly defined line but depends on a number of parameters.

The metastable zone width can be determined by performing a controlled crystallisation experiment. The nucleation can be observed either by the naked eye or more elaborately via a probe using the light reflected by the crystals

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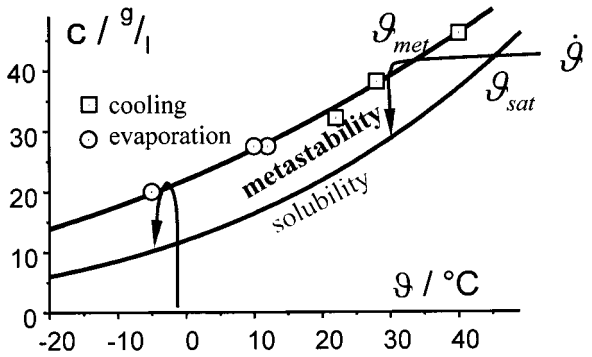

Figure 5. **Width of the metastable region for the cooling and evaporative crystallisation of a steroid. It should be noted that the width of the metastable zone is in first approximation independent of the crystallisation technique.**

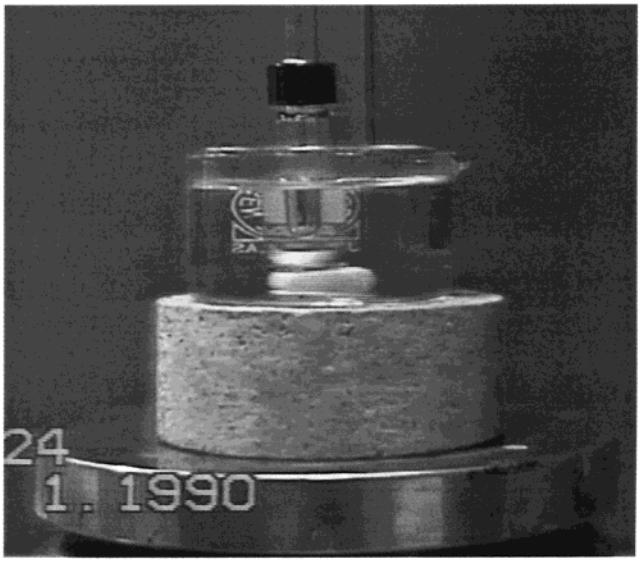

Figure 6. **Experimental setup for determining the width of the metastable region with small amounts of material. The solution is placed into a small vial, and the cooling rate is adjusted by the heat capacity of the water bath. The observation of temperature is by a thermometer placed into the vial, and the observation of the onset of crystallisation is visual.**

formed. In most cases, the detection is facilitated by the fact that the nucleation is an avalanche-type of process: once the nucleation has started, it proceeds with considerable speed.

Figure 6 shows an experimental set-up for determining the width of the metastable region with small amounts of material. The solution is placed into a small, 4 mL-vial. The cooling rate is adjusted by the heat capacity of the water bath. The observation of temperature is by a thermometer placed into the vial, and the observation of the onset of crystallisation is visual. Figure 7 shows a setup, that allows for the semi-automatic determination of the spontaneous nucleation for cooling, evaporative, and drowning-out crystallisations using a sensitive reflected-light probe. The signal from the probe, temperatures, or the loss of mass in evaporative crystallisations are recorded for later evaluation.

In organic systems the width of the metastable zone is (i) nearly independent of the crystallisation technique employed, cf. Figure 5, (ii) at least in technical systems typically a weak function of the velocity with which the system is supersaturated, cf. Figure 8, and (iii) large, values of 10 K are typical, values of up to 50 K are found for sugars.

An influence on the width of the metastable region of considerable importance for organic process development is the (im)purity of the system under consideration. Impurities can hinder the nucleation considerably, thus increasing the width of metastability. This is especially important if early development batches are used for the determination. For a steroid, two different qualities of material were compared, one having an assay of 90% and one having an assay of 97%.[13] Figure 9 shows the width to decrease by a factor of approximately 2 when using purer material. It should be noted that the equilibrium solubility is not influenced by this purity level, as has been shown for this and other substances. It is noted that a correction for the assay has been made; the concentrations are the true ones of the solute.

In addition, the width of the metastable zone can depend on the thermal history of solution, that is, how long and how high it has been heated above saturation and on the number density of foreign particles present in the solution.

In unseeded systems, the formation of crystals is via a spontaneous nucleation that will occur at a certain and usually high supersaturation. For organic systems it is common that during spontaneous nucleation up to 30−50% of the solute dissolved can crash out of solution rapidly, having a detrimental impact on the entire crystallisation process.

Although the metastable zone is not very strictly defined and depends on number of parameters, it gives an indication of the supersaturation or subcooling at which spontaneous nucleation will occur. It is this spontaneous nucleation that is to be avoided in seeding as a relatively large amount of material will crystallise out in an uncontrolled way. The area between the solubility and metastability defines the working area for seeding.

3. Polymorph Spontaneously Formed. The polymorph formed during the nucleation phase of an unseeded crystallisation is the modification that must be avoided during the seeding process. Thus, a discussion of the basic ideas is necessary.

Consider a cooling crystallisation in a system as schematically drawn in Figure 10. First, the solubility line for the stable modification is crossed. Starting here, the stable form is supersaturated, while the unstable form is still undersaturated, that is, crystals of the stable form added to the solution will grow, while crystals of the unstable form will dissolve, and will dissolve as proven in numerous experiments. Upon further cooling, the solubility line for the unstable modification will also be crossed, rendering both forms supersaturated, and crystals of both forms will grow upon addition.

As stated above, although both forms are supersaturated, a certain increase in supersaturation is still needed to induce spontaneous nucleation. The modification nucleated during this spontaneous nucleation is typically the unstable polymorph. This is in essence Ostwald's rule of stages. This rule is—with its exceptions—true both for systems forming polymorphs and solvates.

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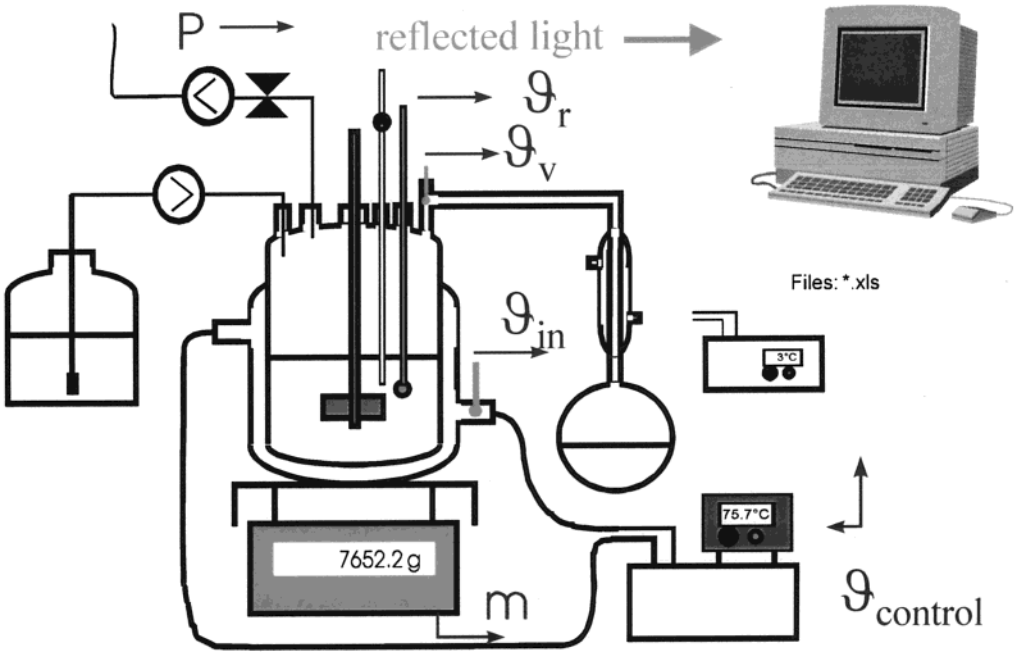

Figure 7. **Experimental setup for determining the width of the metastable region. The setup allows the determination for cooling, evaporative, and drowning-out crystallisations. The onset of crystallisation is followed by a reflecting light probe.**

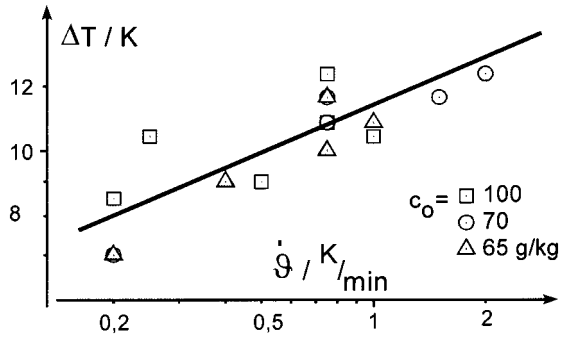

Figure 8. **Width of the metastable region as expressed as subcooling for the cooling crystallisation of abecarnil as a function of the rate of cooling.**

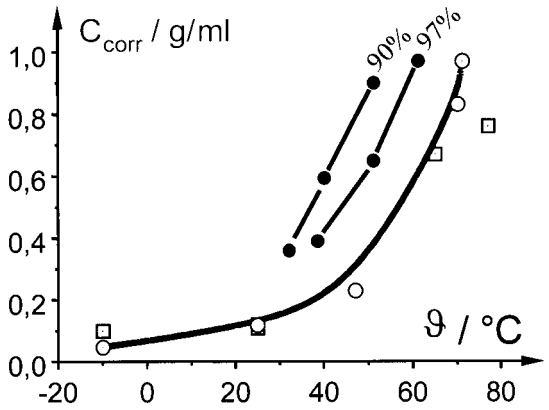

Figure 9. **Solubility and width of the metastable region of a steroid. Both data have been determined for materials with an essay of 90 and 97%. The concentration has been corrected for the different assay. It can be seen that the solubility is independent of the purity level of the material used; however, the width of the metastable region nearly doubles with the increase in impurity level.**

If solvates are involved, the picture becomes more complicated and less well developed. The differences in solubility between solvates can, and generally will be, higher than for polymorphs, cf. Figure 11. It has been pointed out, that the form observed for the cooling crystallisation in solvate-forming systems is determined by the state of association with the solvate determined by the temperature.[14]

When growing an unstable modification, the crossing of the solubility line of this form with the metastability line of a more stable polymorph indicates the control of the polymorphic form to be at least in jeopardy, in essence to have been lost. Consider growing the anhydrate as shown in Figure 11 via cooling below its temperature of stability. Upon further cooling, the solubility line of the now unstable anhydrate will cross the border of metastability for the stable hydrate form, whereupon spontaneous nucleation of this form will occur.

Often the differences in solubility between true polymorphs, Figure 10, is lower than the difference in solubility of an ansolvate and a solvate or between solvates, Figure 11.

Thus, if time permits, the form nucleated spontaneously should be checked as a function of initial concentration or temperature.

4. Transformation of "Metastable" Polymorphs. A metastable polymorph formed via spontaneous nucleation or via seeding will eventually undergo a phase transformation to a more stable one, and finally to the thermodynamic stable form. Most transformations occurring in suspension are solvent-mediated. Transformations in the dry solid state are less often observed. This might be due to the low mobility of the molecules, which is a function of temperature and the difference to the melting point. Thus, only the solvent-mediated transformation is of interest here.

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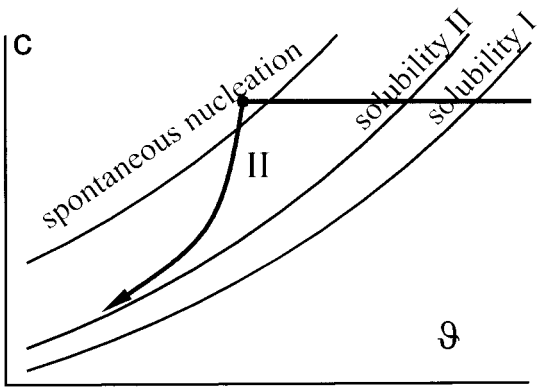

Figure 10. **Schematic representation of the processes during an unseeded cooling crystallisation of a system forming two modifications, I being more stable than II. Spontaneous nucleation will occur after the metastable zone width has been exceeded. The modification initially formed during an unseeded crystallisation is the unstable one, in this case, form II.**

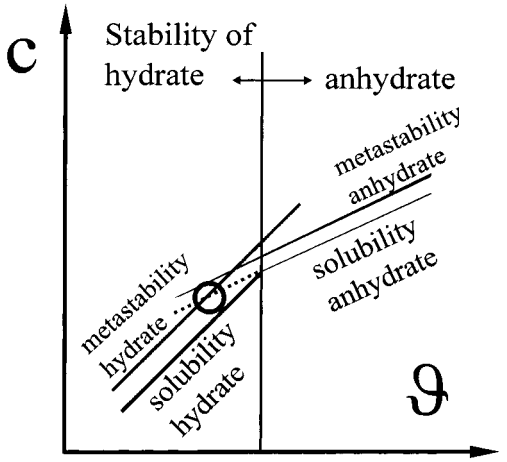

Figure 11. **Schematic representation of the processes during an unseeded cooling crystallisation of a system forming an anhydrate and a hydrate. When growing the anhydrate below its stability domain, the loss of this modification due to spontaneous nucleation of the stable hydrate will occur when the solubility line of the anhydrate crosses the metastable line of the hydrate, as indicated by the circle.**

The kinetics of transformation are governed by the nucleation of the new phase and the transformation process itself. Both processes depend on (i) the degree of which the new phase is supersaturated and thus has a tendency to nucleate and on (ii) the interface kinetics of the dissolution of the old phase, the growth of the new phase, and by the diffusion of mass between the two phases. For the latter, the hydrodynamics of the system plays a role.

Figure 12 schematically shows the fate of a metastable form II initially formed. Form II is initially formed. After an induction time for the formation of nuclei of the stable form I, the transformation to the more stable form I starts and will be completed.

The waiting period for the nucleation of phase I is the window of opportunity to harvest the metastable form II. If the transformation to the more stable form has started, there is no holding back.

The transformation is a thermally activated process thus having a dependence on temperature. Finally, both the

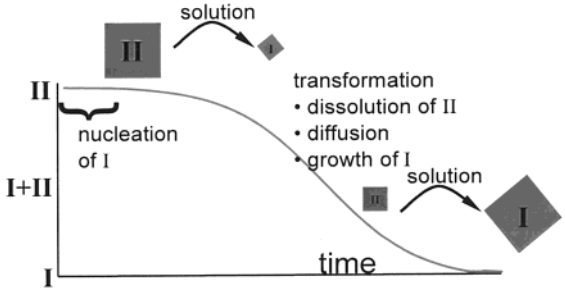

Figure 12. **Development of a metastable modification II initially nucleated and grown during a prolonged keeping of this form in suspension. In a certain induction time, nuclei of the more stable form I are formed. In the subsequent time, the stable form will proliferate at the expense of form II.**

nucleation of the new phase and the dissolution and growth of crystals can be dramatically influenced by impurities in the system.

The presence of solvent is a necessary prerequisite, although a limited amount of solvent might suffice. Transformations were observed to proceed with considerable speed in stagnant slurries with as little as 5% solvent.

Thus, if an unstable modification is desired and crystallised, it is essential to know the stability of the material in a moist state, for example, as a damp filter cake. As especially the nucleation of the new phase is a stochastic process, the stability should be assessed in repeated experiments at different temperatures and with impure and purified material to establish the influence of impurities.

A first approach to this problem can be both a sampling plan during crystallisation and work-up and a prolonged final stirring time.

Seeding Procedures

Definition and Possible Goals. Seeding can be used to control the product crystal modification. This control is effectively exercised during the nucleation phase by adding seeds of the desired form and thus overriding spontaneous nucleation. The addition of seed after spontaneous nucleation has occurred can only induce a solvent-mediated phase transformation. However, this requires the dissolution of the unwanted form. As this step is possibly rate determining, the procedure is not advisable.

The goal of the seeding process is a pure, single polymorphic form of either the stable or unstable form, although seeding and crystallising the stable form is by far the easier task.

An unstable form seeded and grown during the crystallisation phase is prone to a solvent-mediated phase transformation until the product is sufficiently dried. Few attempts have been published to stabilise metastable forms via additives,[15] although this is tempting.

A modification that is lost due to phase transformation during the crystallisation or work-up cannot be obtained via seeding.[16] The point is that the transformation occurs due to the formation of nuclei of the undesired form. It is the

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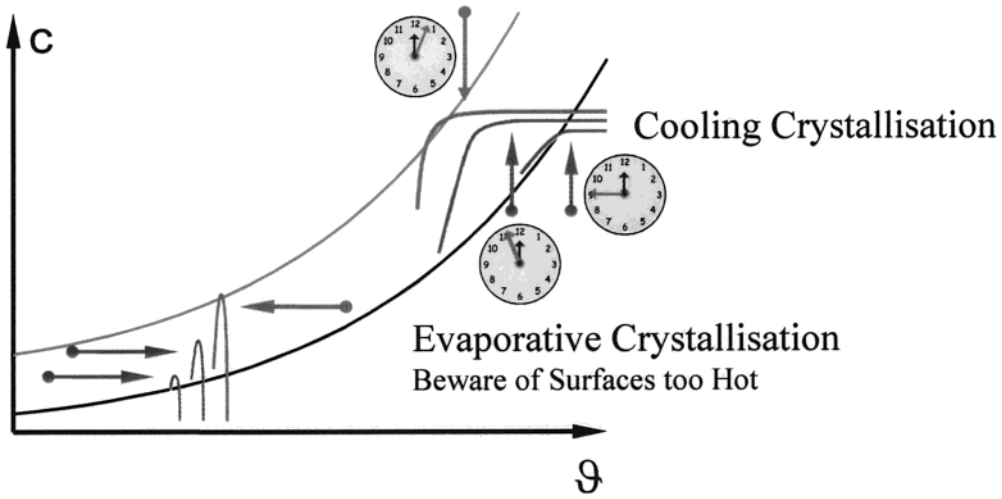

Figure 13. **Window for the addition of seeds for the cooling and evaporative crystallisation. The window is limited by the solubility line and the width of the metastable region. The typical seeding is within the limits of shortly before crossing the solubility line to approximately $^1/_4$ to $^1/_2$ into the metastable zone.**

presence of the undesired from that is to be avoided in this case.

Seeds. The choice of seed crystals is arbitrary; however, for the crystallisation of a drug substance, only crystals of the same substance seem to be allowed. If a stable modification is desired, the seeds can be easily obtained by a solution-mediated phase transformation in any solvent; this transformation will yield the stable form irrespective of the solvent. Of course, this could and will also include the formation of a solvate, if this form is the stable one.

To obtain seeds of an unstable form, the most commonly used techniques for preparation are the use different solvents or a quench-cooling of the melt. If possible, the crop of the previous batch can also be used as seed.

No regulations exist as to controlling the modification via seeding. In fact, its seems to be a question of history and culture if a pharmaceutical company uses seeding intensively or not. However, GMP regulations should be carefully considered while choosing and preparing the seed, for example, by using (i) well-characterised seed material, (ii) material from a related batch or even by preparing the seed from part of the batch to be seeded, and (iii) the smallest amount of seed possible.

As will be shown, the crystals should have a large surface area, so that the use of fines is preferred, for example, in the form of milled material. However, this has also its drawbacks, small particles tend to agglomerate, decreasing the effective surface area.

The surface of a crystal can easily be poisoned, even by exposure to ambient air. This poisoning of the surface causes the growth to be stopped at low supersaturations. In this case the crystals might just act as foreign particles, inducing spontaneous nucleation, possibly of the unwanted form. Thus using a nonactivated seed can jeopardise the entire seeding process. To avoid this, a freshly crystallised seed can be used; however, the need for a concise quick but comprehensive characterisation normally prohibits this procedure. It is better to activate the surface by preparing a slurry, where a dissolution of the outer layer causes the activation. In addition, a slurry in easier to handle than a powder—it can be pumped and does not dust.

The characterisation of the seed should be as concise as possible: two orthogonal techniques are to be preferred, for example, DSC and XRPD. If the seed is not pure, there is the immediate danger of a proliferation of other forms. Not all techniques will show such traces with the same confidence. This is not a problem if the stable polymorph is seeded. If an unstable form is desired, the amount of a more stable form that can be tolerated to be present in the seed is difficult to judge; one can only say via experiments what is too much. For one of the cases discussed below, 5% of the stable form proliferated to 50%.

Window for the Addition of Seeds. The window of seeding with respect to supersaturation is limited from two sides, Figure 13. The lower limit can be chosen within some boundaries. The seed can be added at a low *sub*saturations. In this case, the seed will dissolve until the saturation line is crossed. This dissolution will activate the seed—as preparing a slurry. However, the amount of seed and especially the size distribution has to be adjusted to the rate of supersaturating the system, otherwise, the seed will dissolve before supersaturation is reached.

The upper limit is given by the borderline of the metastable zone which is to be avoided due to the spontaneous and uncontrolled nucleation of large amounts of material it entails. In fact as will be discussed below, seeding has to be done well before reaching metastability.

It should be noted for evaporative crystallisations, that hot surfaces of the heat exchanger might lead to a "partial" dissolution of the seed.

The point of seeding can be derived either from the knowledge of solubility and metastability, fixing the point near to the solubility line and to a maximum of $^1/_4$ to $^1/_2$ into the metastability or by trial-and-error.

Small amounts of seed are added while supersaturating the solution, the point of seeding is reached when the

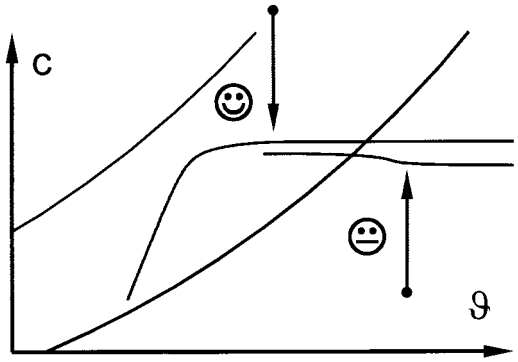

Figure 14. **Pragmatic determination of the point of seeding for a cooling crystallisation. During cooling, seeds are added while observing their fate. In most case, a clear distinction is possible between the dissolution and the growth of the seeds.**

turbidity increases upon seeding. Figure 14 gives a schematic of the latter technique. In practice, a mixture of both techniques will be employed.

Rate of Crystallisation and Amount of Seed. The allowable rate of crystallisation—that is, the rate of cooling, evaporation of solvent, or addition of anti-solvent—is correlated with the amount of seed, more strictly with the active surface area of the seed.

A crystallisation is started at undersaturation, $\Delta c < 0$, and seeding will be around saturation. Two quantities will influence the supersaturation as a function of time, the cooling or evaporation process will increase the supersaturation, $\Delta c\uparrow$, while crystal growth causes a flux of mass out of solution, thereby decreasing the supersaturation $-\Delta c\downarrow$. This push−pull has to be balanced in such a way, that the supersaturation does not reach that for spontaneous nucleation, that is, $\Delta c < \Delta c_{met}$, Figure 15. It should be kept in mind, that metastability is not an exactly defined value, but rather depends on a number of parameters, thus, keeping comfortably away from the value determined experimentally is advisable.

If spontaneous nucleation occurs, the crystallisation is too fast or the seed has been added too late, was insufficient or too inactive. Generally, the amount of seed and rate of crystallisation have to be balanced.

The occurrence of spontaneous nucleation in seeded crystallisations is difficult to detect, as the addition of seeds has already led to the formation of a suspension and the increase in suspension density can either be via the growth of the seed or nucleation. It is thus recommended that the slurry is sampled before completion of the crystallisation, that is, while the system is still supersaturated. An analysis for the modification will reveal the spontaneous nucleation of a second form.

The decrease in supersaturation is given by the mass flux due to growth and is given by $-\mathrm{d}m/\mathrm{d}t \propto A \cdot R$ and thus proportional to the surface area A of the seed crystals present and the growth rate, which is a function of the supersaturation $-R = k\Delta c^n$. By fixing the growth rate, it should be possible to calculate the allowable rate of crystallisation. However, the parameters are difficult to assess, so that experiments to optimise the rate of crystallisation and amount of seed seem

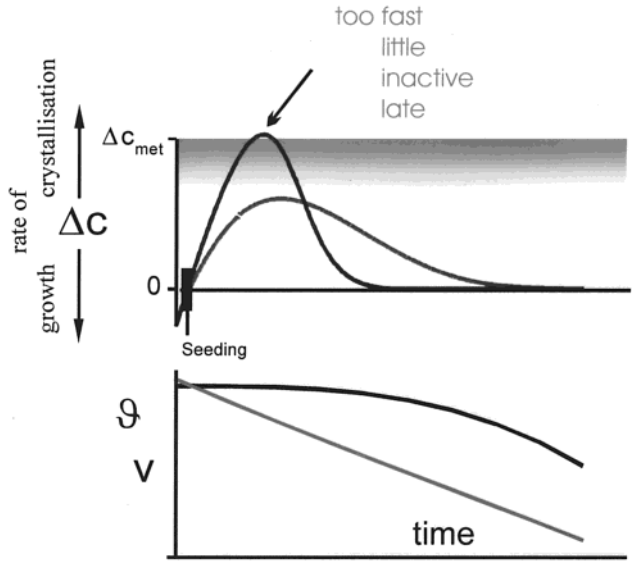

Figure 15. **Development of the supersaturation during a seeded crystallisation. The seeds are added at a concentration around saturation. Due to the small surface area of the seed the growth of the seed depleting the supersaturation will initially not suffice to compensate for the increase due to the continuing cooling or evaporation. The increase in surface area will eventually suffice to decrease the supersaturation by growth faster than it is generated. If this occurs too late, the line for spontaneous nucleation is crossed resulting in an uncontrolled crystallisation.**

easier. Typical values for the amount of seed are given as $m_{seed}/m_{product} \leq 10\%$.

Elaborate schemes have been proposed for adapting the rate of cooling or evaporation for seeded crystallisations to the change in surface area during the crystallisation; the rate has to be low at the beginning of the process and can be increased as the surface increases with mass being deposited on the crystals. These schemes ask for polynomial cooling or evaporative curves. Linear curves are often used as an easy compromise.

Addition of Seed. It has been pointed out that the seed is easily activated by preparing a slurry, that can also be pumped into the crystallisation vessel. Using a slurry also avoids a contamination of the environment due to dusting.

In the case where the purpose of the seeding is only to influence the modification, and not the crystal size, the point of addition is somewhat arbitrary. It is suggested that the seed slurry is added in such a way that it is easily dispersed. Generally, the seed should be added close to the stirrer.[5]

Transfer from Laboratory to Production: Scale-Up Issues. The point of addition of the seed should be a universal constant, as it only depends on saturation. So if concentrations have been unambiguously defined and the solution has been correctly prepared, there is no room for variations. However, for an evaporative crystallisation, the point of seeding has been determined in the laboratory to lay between the saturation at 36.5 g/L and 39 g/L. Transferring the process both to the pilot and production plant gave a complete dissolution of the seed at 39 g/L. Instead, at least a concentration of 42 g/L was necessary to seed the system.

In detail, saturation depends on the assay of the starting material, the concentration, temperature and the composition

Table 1. **Compilation of different seeding techniques as compiled from the literature and private communications**

system	desired form	preparation of seed	technique of seeding
paracetamol	**II, unstable**	**melt paracetamol at 170−180 °C and quench cool the melt**	**scrape material with razor blade into the supersaturated solution at ≤+5 °C and grow at 0 °C. Harvest rapidly and dry thoroughly before storage**
fats	**V, unstable**	**other triglycerides, use of a cryomill**	**add seed upon cooling**
organometallics[17]	unstable form	non-conventional techniques such as "crushing" of material	add seed to solution
abecarnil	**A, unstable**	**cooling crystallization from different solvent**	**add as suspension to slightly supersaturated solution, add sufficient amounts to allow for a rapid cooling and work-up as rapidly as possible**
	C, stable	prolonged stirring of suspension	add suspension to slightly supersaturated solution
steroid	**II, stable**	**prolonged stirring of suspension**	**add as suspension to slightly supersaturated solution**
piroxicam[18]	I, stable	stir form II for >3h	add acid to base ethanolamine in ethanol, heat to complete dissolution, cool and add seed
cimetidine[19]	A, unstable	wash commercially available material with unsaturated solution	cool solution, add seed at A and B supersaturated (> and ≫40%)
	B, stable		cool solution, add seed at A undersaturated and B slightly supersaturated

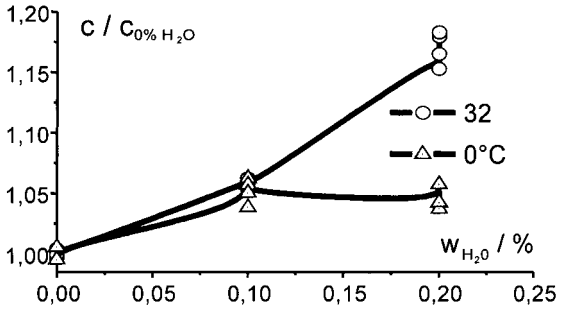

Figure 16. **Variation of the solubility of a steroid in dichloromethane as a function of water content of the solvent at 0 and 32 °C. The solubility is given as change in solubility with respect to the solubility in completely dry dichloromethane. The solubility of water in dichloromethane is ∼0.15 wt %.**

of solvent. For evaporation crystallisations, the temperature is determined by the pressure.

For an evaporative crystallisation at ambient pressure, the saturation concentration depends on the boiling temperature, which is a function of pressure. For the crystallisation discussed above, the boiling temperature of the solvent dichloromethane varies with 60 mK/mbar, that is, an increase in pressure of 20 mbar will change the saturation temperature by ≈1 K! Variations in the pressure can occur via (i) variations in ambient pressure, which are typically 50−100 mbar, (ii) height, when transferring between sites at different elevations, typically 10 mbar/100 m and most important via (iii) a pressure drop in the system or (iv) a pressurisation of the system, 100 mbar is typical for an inert gas blanket. In essence, the saturation temperature can vary dramatically!

Using technical grade solvents can vary the point of saturation. For a crystallisation from dichloromethane the solubility was found to depend on the residual water in the solvent, Figure 16. Dichloromethane can dissolve at room temperature up to 0.15% water. Changing the solvent quality from dry to 0.1% water content changes the solubility by 5%. Taking typical systems, this transforms into a change in saturation temperature of 1.5−3 K.

In essence, the transfer from laboratory and its well-controlled conditions with high quality materials to plant with its technical restraints can have an pronounced influence on the saturation temperature and thus the point of addition of the seed.

Case Studies

A couple of seeding processes for crystallisations which have come to our attention have been summarised in Table 1. The cases set in boldface in the table are discussed in more detail in the following.

Crystallisation of Fats from the Melt.[20] For crystallisations from the melt, seeding strategies have been developed in the laboratory to obtain a certain polymorph of cocoa butter, that is, form V. The goal was the prevention of fat blooming.

As seed crystals, cocoa butter of form VI (!) as well as crystals of other triglycerides were employed. The seed crystals were produced at temperatures <−50 °C using a cryomill.

It was shown that the forms β_1 and β_2 of SOS, the stearic−oleic−stearic acid ester of glycerine, and form VI of cocoa butter were most effective. The authors attribute this to the similarities in the crystal stuctures of the seed crystals and the desired form and the thermodynamic stability of the seed modification.

Metastable Modification II of Paracetamol.[21] Paracetamol is known to crystallise in two modifications, I and II. In addition, a third form has recently been found and its properties elucidated.[22] At room temperature, form II is the

(17) Braga, D. Private communicaton.
(18) Roberson, R. L. (Pfizer Corp.). U.S. Patent 4,582,831, 1986.
(19) Sudo, S.; Sato, K.; Harano, Y. *J. Chem. Eng. Jpn*. **1991**, *24*, 237−242 and 628−632.
(20) For a review, see: Sato, K. Polymorphis of Pure Triacylglycerols and Natural Fats. In *Advances in Applied Lipid Research*; JAI Press: London, 1986; Vol. 2, pp 213−268.
(21) Nichols, G. Paracetamol Form II, The Reappearing Polymorph. In *Proceedings of the 1st International Conference on Polymorphism*; Hinckley, Great Britain, 1999.

stable one, it has been suggested that form II becomes stable at −35 °C.

For the laboratory, a technique to obatin form I was developed using a seeding strategy. The seed crystals of form II were obtained by quench cooling a melt of paracetamol from 170−180 °C. The material was scraped by a razor blade into a supersaturated solution at ≤+5 °C and grown at 0 °C.

The material was quickly harvested and thoroughly dried before storage. Both conditions are due to the fact that small amounts of solvent suffice for a solvent mediated phase transition to the stable form I.

Stable Polymorph II of a Steroid.[13] The steroid is known to crystallise in three modifications, named I, II, and III. All modifications are thermodynamically close, and no interconversion is observed in the solid state. Using enthalpy−temperature diagrams, II was found to be the stable form between −10 and +60 °C. The data were corroborated by storing mixtures of forms II and III at room temperature. A transformation to II indicates this form to be the stable one. Form I was observed to transform above 0 °C either into form II or III, so that it was not added in these experiments.

The unseeded cooling crystallisation yields for most solvents the unstable form III. In suspension, this form eventually transforms into the stable form II; however, the kinetics are slow.

It was decided to use the stable form II as solid-state form of the drug substance. Thus, a crystallisation technique had to be developed, yielding pure form II.

The solubility of the steroid was measured as a function of temperature, Figure 9. It can be seen that only few points were gathered for the solubility.

Using the few data gathered on the system suggested using a cooling crystallisation, starting at ∼80 °C and ending at room temperature, ensuring a sufficient yield and high purification. For the point of seeding, preparation of seed and rate of cooling, it was observed that spontaneous nucleation occurs at high subcoolings, ΔT > 10−20 K, so that the addition was set 10 K below the point of saturation that was estimated from Figure 9.

The amount of seed was set to 0.5%, with the seed activated by preparing a slurry at room temperature. The rate of cooling was set to 0.25 K/min, a relatively slow rate.

The point of addition of the seed was varied slightly for several crystallisations always yielding the desired form II. Further experiments showed that the crops of the crystallisation process could be reused for further seeding. With these few experiments, the seeding technique was transferred to pilot plant, where it successfully worked.

However, the metastability was determined especially as a function of the purity of the starting material, Figure 9. As can be seen from Figure 17, the point chosen for the seeding is close to the metastability of a pure material. Thus, the points of seeding were redetermined by the trial-and-error method as shown in Figure 14. Not astonishing, the points lay closely to the solubility line.

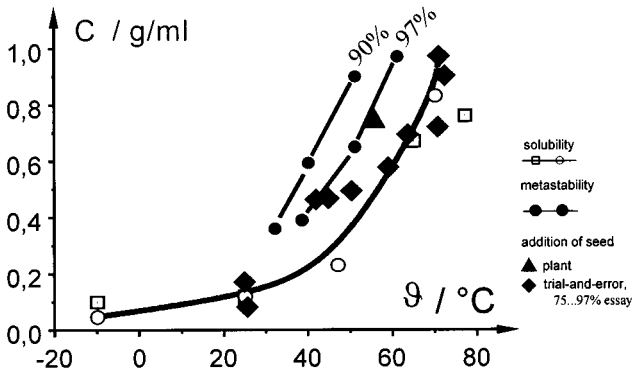

Figure 17. **Points of seeding as developed (i) heuristically and (ii) in more scientific way. The data are given for different materials with a purity of 90 and 97%. The data can be compared with the width of the metastable zone for the different materials and the equilibrium solubility, that is the same for all materials.**

In retrospect, the seeding process was only successful due to the fact that relatively impure material was used, an increase in purity would have resulted in an spontaneous nucleation with the conditions used. It is thus advisable to determine the point of seeding by the trial-and-error method, if no reliable saturation concentration data are available.

Second, by choosing the stable form, seeding a system becomes self-stabilising. A certain amount of metastable form precipitated will undergo a solvent mediated phase transformation to the stable one and thus vanish.

Metastable Modification A of Abecarnil.[23] Abecarnil is a β-carboline derivative known to form three modifications, A, B, and C. The forms can easily be distinguished by their external shape. A quantification is possible via XRPD and DSC. Measurements of solubility of all three modifications, Figure 2, show the C form to be the monotropic phase stable at all temperatures. A and B form an enantiotropic pair, with A more stable at temperatures below 60−70 °C. These findings are corroborated by thermoanalytical data, Figure 5.

The unseeded crystallisation of abecarnil follows Ostwald's rule of stages, spontaneous nucleation yields one of the unstable forms. From alcohols A is obtained, while B is obtained from esters. The solvent-mediated phase transformation B to A depends on impurities in the system, see below. The transformation to the stable C form is much slower. A phase transformation in the completely dry state was not observed.

The history and problem of the crystallisation of abecarnil can be summed up as follows. The unstable B modification was first chosen as solid-state form of the drug substance because this form spontaneously nucleates from isopropyl acetate—the solvent appropriate for crystallising of this substance (abecarnil is an isopropyl ester). During early development, B was the only form obtained from this solvent and found to be *stable* in suspension, the transformation to A needed more than 1d. However, with the establishment of a new synthetic route, the product became purer, especially

(22) Szelagiewicz, M.; Marcolli, C.; Cianferani, S.; Hard, A. P.; Vit, A.; Burkhard, A.; von Raumer, M.; Hofmeier, U. Ch.; Zilian, A.; Francotte, E.; Schenker, R. *J. Therm. Anal. Calorim.* **1999**, *57*, 23.

(23) Beckmann, W.; Nickisch, K.; Budde, U. *Org. Process Res. Dev.* **1998**, *2*, 298.

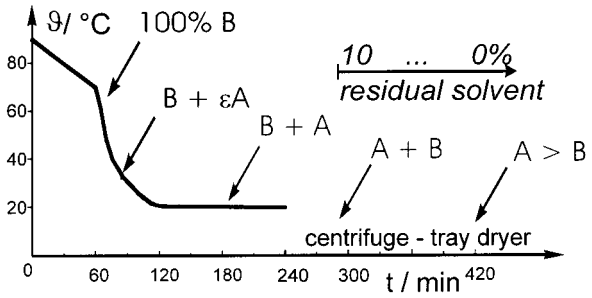

Figure 18. **Modification of abecarnil of the new synthetic route during the nucleation and subsequent crystallisation phase and during work-up. The transformation occurred even for material having as low as 5−10% residual solvent.**

the content of an acidic component decreased. This entailed the product to transform within 2−3 h into A.

An analysis of the process in the plant showed that during nucleation, the B modification was still formed—with no detectable amounts of any of the two other modifications. However, the transformation to form A started already during the cooling phase and continued throughout the solid−liquid separation and drying until the product was completely dry, Figure 18. It was shown that the transformation occurs even for material having as low as 5−10% residual solvent content. The transformation is not necessarily completed, instead the transformation results in mixtures of A with up to 40% B.

In essence, the ability to obtain the B modification reproducibly was lost. Pure B formed during the spontaneous nucleation—but started transforming into A in considerable quantities already while cooling the solution.

The search for and consequently use of additives to inhibit the nucleation of A or to stabilise B was dismissed for regulatory considerations. A switch to the C modification was also dismissed, this form had an entirely different habit. Contrarily, the A form had been shown to have an equivalent bioavailability and it is also structurally close to B. Thus, A—the second metastable modification—became the alternative.

The task was to develop a technique to crystallise the A modification from isopropyl acetate with no detectable amounts of either C or B modification. This meant avoiding the intermediate formation of B during spontaneous nucleation via seeding whilst being fast enough to avoid a transformation to C.

The seed was prepared by crystallising abecarnil form methanol, a solvent yielding the A modification upon spontaneous nucleation. It showed no traces of either B or C modification both in DSC and XRPD. The crystals are thin long needles, having a large surface area. In addition to using the crystals as grown as seed some batches were also micronised to a mean size of 3−5 μm and then used as seed.

For the development of the seeding process, the addition was set to be made at a slight supersaturation of the sought for A form. Also the seed was activated by forming a slurry. A comparison of dry and activated seed using 1% of seed and a cooling rate of 0.5 K/min had shown the occurrence of B when using dry seed, a clear indication for spontaneous nucleation. With the slurried seed, no formation of B was observed for these conditions, for more details see below.

The solvent used to slurry the seed was isopropyl acetate. Its amount was chosen in such a way, that 10−20% of the seed dissolved.

The amount of seed, rate of cooling, type of seed, and speed of work-up were optimised. The amount of seed was

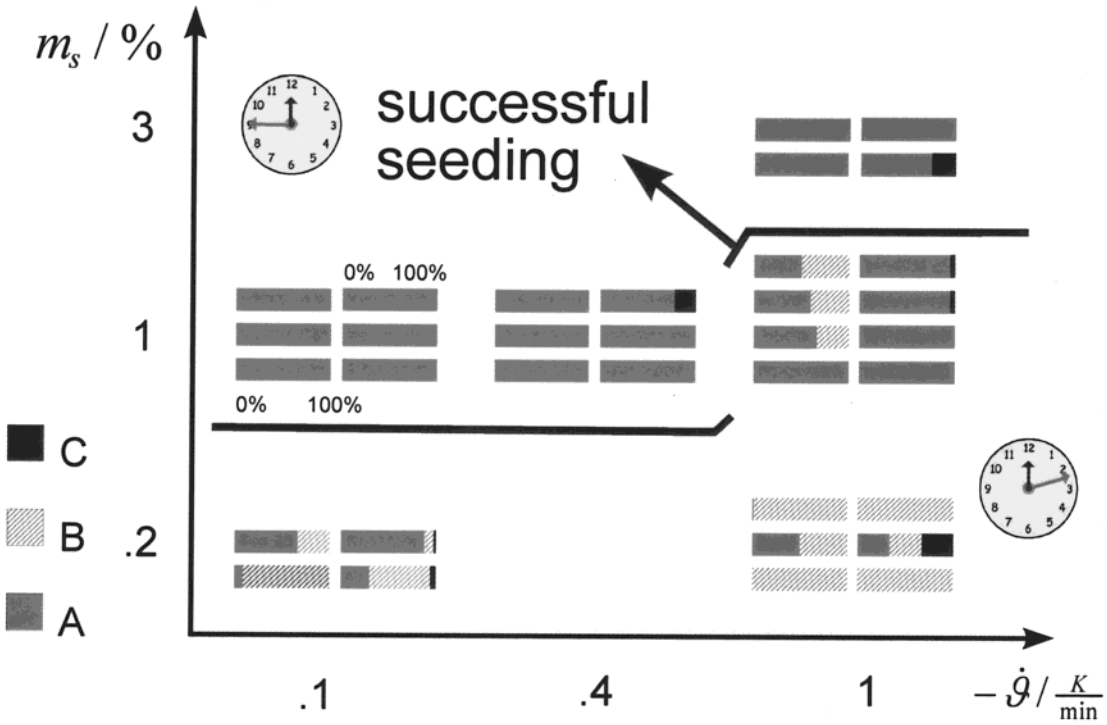

Figure 19. **Polymorphic composition of seeded crystallisations of abecarnil using amounts of seed of m_s = 0.2−3% as referred to the amount of abecarnil in solution and cooling rates of $-\dot{\vartheta}$ = 0.1−1 K/min. The fraction of the non desired forms B and C are shown. The conditions for successful seeding are designated by the diagonal line.**

varied between 0.1 and 3% seed, and the rate of cooling was varied between 0.1 and 1 K/min. A number of experiments were carried out for each combination, varying also the type of seed. For each point, the modification was determined after work-up. The results are summarised in Figure 19. It can be seen that a considerable amount of seed is necessary for high cooling rates to obtain pure A modification; otherwise the decrease in supersaturation is insufficient to avoid spontaneous nucleation of B. A diagonal line separates the border for successful seeding.

A cooling rate of 1 K/min constitutes a *crash* cooling, only permissible because of the high amounts of seed added. However, for slow cooling, a tendency for the formation of C was observed; thus, the crash cooling was chosen.

The crop from the seeded crystallisations could be reused as seed for further batches. However, when a particular batch that contained A plus 5% C was used, A with ≈50% C modification was obtained after work-up. This underlines the importance of a careful determination of the modification of the seed.

The parameters chosen for the crystallisation are thus 3% seed, added as slurry at slight supersaturation of A, and a cooling rate of 1 K/min.

Conclusions

In summary, for seeding to be successful, some points are worth considering:

The polymorphism of the system should be understood and

it must be known if a stable or unstable form is sought. If an unstable form is desired, it should be known how far this is apart from the stable one; if the solubility line of the unstable form crosses the metastability line for a more stable form there is no chance for seeding.

The fate of the forms during crystallisation and work-up should have been elucidated: that is, the possibility for a transformations should be known and possibly also its rate. A form lost during crystallisation and work-up will be difficult to obtain via seeding.

The development of the seeding process is facilitated by information on the solubility and metastability.

As far as seeding is concerned, seeking the stable form is the easiest task, metastable forms require more efforts to be spent characterising the system in terms of solubility, phase-transformation behaviour, and in the properties and characterisation of the seed. All characterisations should be as concise as possible.

To avoid approaching too close to the boundary of the metastable zone it is necessary to optimise the seed quantity and the rate of crystallisation.

These few peaces of information and reflections on the system should suffice to succeed in controlling polymorphism via seeding—to *succSeed*.

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