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POLYMORPHISM – INTEGRATED APPROACH FROM HIGH-THROUGHPUT SCREENING TO CRYSTALLIZATION OPTIMIZATION

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Abstract

Crystal structure (polymorphism) as well as crystal shape (morphology) and size have a huge practical and commercial impact on active substances all the way from research to manufacture of the final product. For an optimal development process, it is important to have an integrated approach to these issues ranging from a systematic polymorphism screening to a controlled scale-up of the crystallization process. The polymorphism program has to be tailored according to the development stage. Particularly suitable for an early development stage is a high-throughput polymorphism screening, which is the basis for a more thorough investigation if the product proceeds further in development. Such a comprehensive polymorphism investigation involves further crystallization experiments and extensive physicochemical characterization of the various forms.

In this article the high-throughput polymorphism screening method that we have developed is described. Using carbamazepine as an example, the power of this high-throughput polymorphism screening system is demonstrated. Not only were all published forms found, but also new forms were identified.

In the second part of the article, important considerations for crystallization optimization are discussed, again using the example of carbamazepine.

Keywords: carbamazepine, crystallization, high-throughput, polymorphism, screening

Introduction

Polymorphism is the ability of a compound to crystallize in more than one distinct crystal structure [1]. The probability that a particular drug substance can exist in different solid forms is high. Frequently, it is quoted that about half of all small organic molecules can exist as polymorphs or solvates [2]. In our experience this number is closer to 80%.

These modifications or polymorphs have different physical and chemical properties. From the pharmaceutical point of view, bioavailability, processability and stability are influenced by the existence of polymorphs. In order to avoid undesired changes during the production process or during the product lifetime, it is of the utmost importance to identify and control the polymorphic behavior of any drug [3–5].

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Polymorphism screening should only be seen as one step in long process involving solid-state issues, however (Fig. 1). Often new substances showing pharmaceutical activity are weak acids or bases, which have very low water solubility. In such cases, it may be preferable to market the drug as a salt form with better water solubility. In a salt screening process, the optimal salt is chosen, taking parameters such as solubility, hygroscopicity, crystallinity, ease of production, etc. into account. Then, a careful polymorphism screening has to be performed either for the original molecule or for the selected salt(s) to reveal all relevant polymorphic forms. Patenting these forms is highly advisable in most cases. Based on the physicochemical properties of the various forms, which were determined as part of the polymorphism screening and supplemented by biological tests, the most suitable form for further development is selected. Then methods, which may be based on, e.g., differential scanning calorimetry (DSC), X-ray diffraction (XRD), Raman spectroscopy, etc., have to be developed to determine the polymorphic purity in both the drug substance (DS) and drug product (DP). If the form that was chosen for development is a metastable form, it is imperative to determine the kinetic stability of the latter. In the polymorphism screening, the form that was finally chosen was probably produced in an inefficient, industrially non-feasible way. The next important step is therefore crystallization scale-up of the desired form, optimizing yield, morphology, crystal size, costs, time, and environmental impact of solvents, etc. If a metastable form is chosen for production, it may be necessary to monitor the crystallization on-line to determine the appropriate endpoint. And finally, quality control (QC) of both drug substance and drug product is crucial.

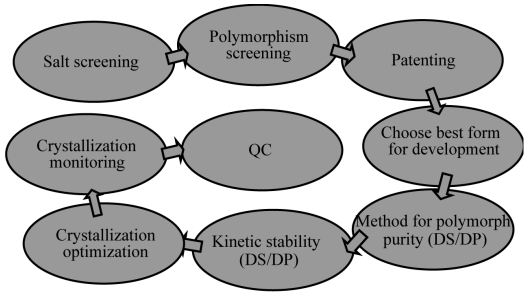

Fig. 1 Solid-state issues in the course of the development cycle

In this article, we highlight the most relevant aspects of polymorphism screening and crystallization optimization. In particular, our recently developed high-throughput screening will be discussed.

Polymorphism screening

It makes sense to perform polymorphism screening along a well-defined sequence. Typically this involves characterization of the substance that is used for screening by DSC, thermogravimetry coupled to infrared spectroscopy (TG-FTIR), XRD, Raman

spectroscopy, magic-angle spinning (MAS) NMR, solubility and so forth. This is often followed by hot-stage optical microscopy or hot-stage Raman microscopy, which can give extremely valuable information for substances that are thermally stable up to their melting points. The next step is often a search for hydrates by dynamic vapor sorption (DVS) or water/solvent slurry methods. Normally crystallization experiments are by far the most labor-intensive part of the screening, requiring the use of many solvents and solvent mixtures that show a large difference in appropriate physicochemical properties as well as various crystallization methods. Finally, the polymorphs that have been found are characterized.

Since polymorphism does play such an important role in the development of a drug, it is clearly advisable to do a polymorphism screening as early as possible in the drug development process. On the other hand, it certainly doesn't make economical sense to carry out a comprehensive study very early in the development as the probability of such a molecule making it to market is small. It is therefore most sensible to carry out polymorphism programs of various depths adjusted to the particular development stage.

As mentioned above, crystallization experiments are the most time (and substance) consuming steps. With this in mind, we developed a high-throughput screening (HTS) system, with the aims of (*i*) decreasing the time needed to perform crystallizations, (*ii*) decreasing the amount of substance needed and (*iii*) increasing the diversity of crystallization conditions simultaneously (patent application pending). This means, obviously, that miniaturization, automation and use of parallel techniques are required.

Results of the HTS are the basis for more complete polymorphism studies if the product proceeds in development. Such a comprehensive polymorphism investigation involves further crystallization experiments and extensive physicochemical characterization of the various forms, including the investigation of their thermodynamical relationships as a function of temperature.

High-throughput polymorphism screening (HTS)

Critical elements of an HTS include the choice of crystallization techniques, handling procedures, detection methods, and data processing as well as storage. Perhaps the most important issue is the optimal selection of crystallization techniques and conditions, however, as it is very easy to perform thousands of crystallizations and always end up with the same solid form!

Crystallization techniques for screening

After a thorough comparison of the various options, crystallization from solution by controlled evaporation of the solvent together with suspension experiments have been chosen as the major crystallization methods. Furthermore, both wet and dried crystals are analyzed, i.e., desolvation of solvates is used as the third method for generating new polymorphs. These techniques enable the investigation of both thermally stable and unstable substances, which are not accessible for screening using thermal

methods. The use of a number of solvents and solvent mixtures creates different nucleation and crystal growth conditions so that stable as well as metastable forms can be produced. In addition, the stability of hydrates and solvates as a function of water or solvent activity can be investigated by equilibration in solvent mixtures.

Suspension experiments play a key role, as they are most likely to lead to the thermodynamically stable form [6]. And not finding the most stable form is probably the worst outcome that can happen.

Suspension and evaporation experiments are easily miniaturized and require only approximate solubility data. 96-well microtiter plates made from quartz with PTFE seals were chosen to make the system leakproof and to be compatible with a majority of solvents. The plates can be heated and shaken. For evaporation experiments, the sealing was modified in order to allow a flow of N_2 to pass through the individual wells.

Apart from the three major crystallization methods, other techniques may be used, such as precipitation, vapor diffusion, cooling of saturated solutions, etc.

Solubility

As mentioned above, the approximate solubility of the substance in the solvents and solvent mixtures that are to be used has to be known in order to perform the crystallization experiments in a meaningful way.

Solubility of the substance was determined by a UV/VIS assay. The method is applicable to molecules showing sufficient UV/VIS absorbance.

For each solvent or solvent mixture, 100 μL of a saturated solution was prepared by adding an excess of drug substance to the particular solvent. The suspensions were equilibrated for about 16 h and filtered by centrifugation (9000 rcf, filter units: Millipore Ultrafree-CL, 0.22 μm PVDF membrane).

Ten solvents were tested at once. A quartz 96-well plate was prepared by filling 60 μL of 1,4-dioxane in each well. 30 μL of the respective filtrate was transferred to each of the first ten wells of the first row. 30 μL of a standard solution (about 50 mg mL^{-1} in 1,4-dioxane) was added to the following well. The last column was used as a blank.

The solutions of the first row were subsequently serially diluted by transferring 30 μL of each solution to the next row.

Finally, the absorbance was measured between 220 and 360 nm against pure 1,4-dioxane using a multi-wavelength spectrophotometer (μQuant, BIO-TEK Instruments). The solubility was determined by selecting one or two dilutions with suitable optical density and comparing them to the standard.

Characterization

Out of the many possibilities (NIR, IR, XRD, DSC, synchrotron-XRD, etc.), Raman microscopy was chosen for the characterization of the crystals. This technique allows the characterization of crystals of diameters down to some micrometers; i.e., some picograms of crystals are in principle sufficient to determine its solid form. This is many orders magnitudes less than what is needed for XRD. Raman spectra are col-

lected directly in the micro-titer plate. No additional preparation step is needed. For suspension experiments, spectra are collected both in the presence and after the evaporation of the solvent. Thus labile solvates can be easily detected. Spectra are collected automatically in a predefined grid for suspension experiments. For evaporation experiments, optimal data quality is obtained by manual spectra acquisition whereby crystals are visually selected for Raman analysis. Automatic spectral analysis allows a rapid division of Raman spectra into classes based on their spectral similarity.

Another advantage of the spatial resolution of Raman microscopy is that mixtures of several forms within the same well are normally easily identified. Further information that can be gained by visual inspection, such as crystal morphology, may also be very helpful.

Example of high-throughput polymorph screening using carbamazepine as a model substance

Properties of carbamazepine

Carbamazepine (CBZ, Fig. 2) was chosen as a model substance to demonstrate the efficacy of the HTS since it is both well-investigated and exhibits many different forms and therefore represents a challenging example. It is a well-established drug used in the treatment of epilepsy and trigeminal neuralgia. Several polymorphs as well as two pseudo-polymorphs (dihydrate and an acetone solvate) of CBZ had previously been identified in the literature [7–12, 14]. CBZ of highly pure form III obtained from Novartis was used

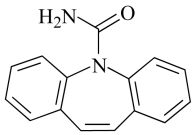

Fig. 2 Carbamazepine (CBZ)

for all experiments. In this article we use the nomenclature of Griesser [7].

Hot-stage Raman microscopy

Before carrying out crystallization experiments from solvents, it is advisable to perform melt crystallizations. There are examples in literature, where certain forms (e.g., paracetamol form III) can only be produced by melt crystallization [15, 16]. Hot-stage Raman microscopy of CBZ produced forms I and III.

Suspension and evaporation experiments

Experiments with CBZ form III, i.e., the thermodynamically stable form at ambient temperature, were carried out in 43 solvents and solvent mixtures. For suspension experiments, slurries of CBZ in 100 μL of solvent were equilibrated for 20 h at room temperature. The suspensions were analyzed in the presence of the solvent and after complete evaporation of the solvent. Evaporations were started with 100 μL of solu-

tion. They were carried out in both a nitrogen atmosphere with two different evaporation rates as well as in the free (ambient) atmosphere. For the complete set of suspension and evaporation experiments, less than 1 g CBZ was needed.

Data acquisition

Raman spectra of the crystals obtained by suspension experiments were collected automatically using a predefined grid. Four different positions per well were sampled in the presence of the solvent and three different positions after evaporation of the solvent. The automatic mapping of the plate comprising 50 measurements was completed within 45 min. Raman spectra of the crystals obtained by evaporation were measured manually by visual selection of the crystals. Three crystals per well were investigated.

From our experience, Raman spectroscopy is an excellent method to discriminate among different polymorphs and pseudopolymorphs of substances. We found that failure to discriminate between solid forms is similar for Raman and X-ray diffraction, provided that the Raman spectra are analyzed very carefully.

Data analysis

All data acquired during the screening experiment were stored in the 'HTS data manager', a central database which was developed by Solvias. For each well of the plate, the database comprises results of the Raman microscopy measurements including spectra, pictures and acquisition parameters, as well as information about the experimental conditions, e.g., solvents, temperature and humidity.

Based on their spectral similarity, the Raman spectra were divided automatically into 12 different classes by the computer program 'Peak Compare' developed by Solvias. The program automatically subtracts the contributions from residual solvent. Spectra are compared to each other on the basis of peak position and reference spectra are defined for each class of spectra. Alternatively, spectra can also be compared to predefined references. All relevant parameters for spectral comparison can be manually adapted.

Reference spectra for each class are also stored in the 'HTS data manager'. The result of the automatic evaluation is shown in Table 1. The program defines a reference spectrum for each class. Visual inspection of the reference spectra identified class 7 as a mixture of the classes 1 and 6.

Crystallization experiments with CBZ on a larger scale and subsequent analysis by Raman spectroscopy and X-ray powder diffraction allowed the assignment of the six classes of polymorphic and pseudopolymorphic forms described in the literature [7–12, 14] as shown in Table 2. The recently published new form IV [14] is most probably identical to form IIa. The single crystal data from Lang *et al*. [14], which are in fact different from previously published single crystal data, were converted to powder XRD data by using the program Powder Cell 2.4 (Federal Institute for Materials Research and Testing, Berlin, Germany). The findings reveal that it is identical to form IIa [17]. Most of the new classes were tentatively assigned to solvates. No new form that is more stable at room temperature than form III was found.

Table 1 Part of the matrix of carbamazepine experiments with identified forms

Solvents/mixtures	Crystallization conditions				
	Suspension wet state	Suspension dry state	Very slow evaporation (N_2)	Slow evaporation (N_2)	Fast evaporation (free atmosphere)
Acetic acid	3	3	8	8	8, 3
Anisole	1	1	8	8	8
Acetone	12	1	1	1	2, 11
Dimethylsulfoxide	4	–	–	–	–
Ethanol	1	1	8	8	2
N,N-dimethylformamide	1	1	–	8	8
1,4-dioxane	5	5	9	9	8, 9
Methanol	1	1	6	6, 11	2
Toluene	1	1	8	8	8
Isopropylether	1	1	8	8	8
Tetrahydrofuran/water 1:1	2	1	6	6	2
N-methylpyrrolidone	1	1	–	8, 10	–
Ethanol/water 1:1	2	1, 7	6	6	2
...	...	...	...	...	...

Table 2 Correspondence between classes and forms described in the literature

Classes	Forms
1	III [7–9, 11, 12], beta-form [10], monoclinic form [11]
2	Dihydrate [7–10]
3	New form: molecular compound
4	New form: DMSO solvate
5	New form: dioxane solvate
6	IIa [7]; II [9, 12]; IV [14]
7	Mixture of IIa and III
8	IIb [7], II [8], alpha-form [10], trigonal form [11]
9	New form
10	New form
11	I [7–9, 11, 12], gamma-form [11], triclinic form [11]
12	Acetone solvate [7, 10]

Further investigations will be necessary to characterize the new forms. Figure 3 shows a comparison of the Raman spectra of the forms I–III.

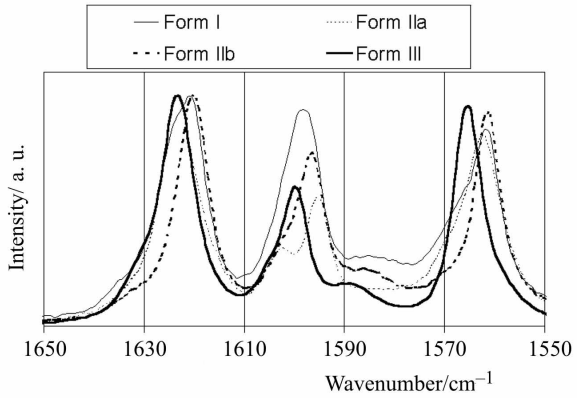

Fig. 3 Selected region of the Raman spectra of some of the identified forms of carbamazepine

The results demonstrate the suitability of HTS to discover the relevant polymorphic forms. As mentioned above, further investigations, such as elucidation of the thermodynamic relationships between the forms [18–24], extensive physicochemical characterization of all forms, etc., would subsequently be required. Data from literature show that form III is the thermodynamically stable form in the technically relevant temperature range [9, 11, 13, 25–27].

Crystallization optimization

We again chose CBZ as a model substance to elucidate certain issues with respect to crystallization scale-up. Crystallizations were performed in a heat-flow calorimeter, since it offers many convenient features, such as the ability to work with reasonably large quantities of about 100 g, excellent temperature control and reproducibility of conditions, and possibilities for on-line measurement of turbidity, particle size distribution, and IR and Raman spectra.

A typical aim of crystallization optimization is to produce form III in an efficient way using only Class 3 solvents. If CBZ form III is dissolved in a solvent by heating and recrystallized by cooling, the form obtained depends on the solvent (Table 3). Under these conditions (cooling rate 2 K min^{-1}, stirring rate 300 rpm, no slurry ripening), form III cannot be obtained with the Class 3 solvents used.

Table 3 Forms obtained by spontaneous nucleation: CBZ is completely dissolved by increasing the temperature and recrystallized by lowering the temperature (2 K min^{-1})

Solvent	ICH Q3C Class	Concentration/g 100 mL^{-1}	Modification
MeOH	2	15–60	III
Acetonitrile	2	8	III
Dioxane	2	20	Solvate
Toluene	2	0.8	IIb**
THF	3*	10	IIb**
Ethanol 96%	3	4.5	Mixture of IIb/dihydrate
Acetone	3	3	Solvate
Ethylacetate	3	3	IIb**
Ethylmethylketone (MEK)	3	6–12	IIb**

*ICH Q3C (M): it is recommended that THF be placed into Class 2
** Samples contain small (1 to 3%), non-stoichiometric amounts of solvent [28], small differences are visible in powder XRD patterns

Table 4 Influence of cooling rate and concentration on the form obtained at 20°C by spontaneous crystallization without slurry ripening. Solvent: MEK, stirring rate: 300 rpm

Cooling rate/K min^{-1}	Concentration/g 100 mL^{-1}		
	12	9	6
2.0	IIb	IIb	IIb
1.0	Mixture of III and IIb	IIb	IIb
0.5	III	Mixture of III and IIb	IIb
0.1	III	III	III

Table 4 shows the influence of concentration and cooling rate on the form which is obtained in MEK. In line with Ostwald's Rule of Stages [29], the thermodynamically more stable form is preferentially obtained under conditions of low supersaturations, i.e., at low cooling rates. One has to bear in mind, however, that spontaneous crystallization is influenced by many factors that can hardly be controlled, such as small impurities and hydrodynamic effects. This can lead to mixtures of forms (Table 4) and poor reproducibility. Therefore crystallizations using seeding instead of spontaneous nucleation are preferred in most cases.

The key for a good seeding strategy is the precise knowledge of the metastable zone with of all relevant forms in the solvent of choice [30–34]. In Fig. 4, the metastable zone width of both forms IIB and III in MEK is depicted.

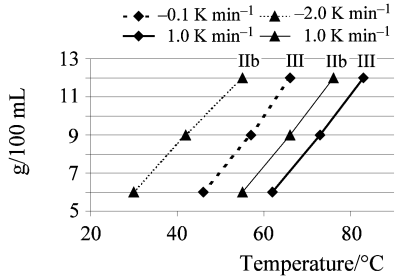

Fig. 4 Solubility (solid lines) and supersolubility (broken lines) of CBZ forms IIb (triangles) and III (diamonds) in MEK

Using these data, form III was efficiently produced in MEK by adding seeds at 57°C to a solution containing 60 g L^{-1} CBZ being cooled at a rate of 2 K min^{-1}. This process is about 20 times faster and much more reliable than, e.g., producing form III by spontaneous nucleation and a cooling rate of 0.1 K min^{-1} (Table 4).

Conclusions

Solid-state properties have enormous significance for a pharmaceutical product and it is imperative that they are dealt with in an integrated procedure all the way from salt screening to quality control. One step of particular importance is certainly polymorphism screening. For economic and practical reasons, the nature and extent of the screening should be matched to the stage of development of the drug. In an early stage, where only a small amount of substance is available, an HTS is particularly appropriate. At later development stages, such a screening should be complemented by other crystallization methods and a thorough physicochemical characterization of all products.

Significant commercial advantages can also be gained by designing good crystallization processes. Precise knowledge of physicochemical properties of the various forms is a prerequisite for that.

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