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SOLID-STATE OF PHARMACEUTICAL COMPOUNDS
Impact of the ICH Q6 guideline on industrial development

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Abstract

This article summarizes the different steps needed for a proper design and monitoring of the solid-state in pharmaceutical industry in order to fulfill the requirements of the guideline dealing with polymorphism of the International Conference of Harmonization.

Keywords: amorphous forms, design of the solid-state, hydrates, kinetics, monitoring, pharmaceuticals, physical analytical methods, polymorphism, quantification, solid-state properties, solid-state transformations, solvates, thermodynamic relationships

Introduction

The chemical industrial development in pharmaceutical industry is faced with the acceleration of the development time of new medicines and with harmonization guidelines which are required by health authorities for worldwide registration.

Polymorphism is the ability of a substance to crystallize into different crystalline forms. These crystalline forms are called polymorphs or crystalline modifications. Polymorphs have the same liquid or gaseous state but they behave differently in the solid-state. The amorphous state is characterized by solidification in a disordered, random manner, structurally similar to the liquid-state. The expression pseudo-polymorphism applies to hydrates and solvates. These three types of solid phases are considered under the term 'polymorphic behavior' in this article. Review articles about polymorphism and relevance [1–8].

Intrinsic properties of a new drug substance candidate are its pK_a, its lipophilicity expressed by log*P* and its intrinsic chemical stability in solutions. Poor physical properties of substances may be changed by using the salt formation in case of acidic or basic compounds and by considering different polymorphs. Formulations are developed to improve solid-state properties and in certain cases affect the solid-state of the drug substance in the drug product.

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The 'polymorphic' behavior of organic substances is driven by thermodynamic and kinetic factors. Therefore several solid phases can coexist. Since the properties of the solid-state may be extremely relevant for the quality of drug products, the International Conference for Harmonization (ICH) requires a polymorphic study of each new active ingredient with a three-step decision tree starting with detection of different solid phases, followed by the characterization of the different solid phases and if the properties are relevant for the drug product quantitative methods and specifications have to be developed for the drug substance and even for the drug product (ICH guideline Q6A [9]).

The most relevant property for health authorities [10–12] is the bioavailability and the bioequivalence of formulation not always correlated with polymorphism, the stability and the robustness of the processes.

The processing of the drug substances and drug products involve solvent(s), temperature and pressure changes as well as mechanical stress and different solid phases may coexist in the drug product. Organic substances show supersaturation behaviour and unstable solid phases which should not exist in defined temperature, pressure and humidity may behave like stable forms.

These solid metastable phases obtained outside their domains of stability will convert into the thermodynamic stable forms at given temperatures, pressures and relative humidities. These conversions driven by thermodynamic are governed by kinetic and are influenced by impurities, particle size, crystal defects, presence of seeds.

Design of the solid-state is the best choice of the salt candidate and the polymorphic form as early as possible in order to avoid delays due to new development, bioequivalence studies and upscale has to be taken into consideration since synthetic processes will be optimized from the first mg material to the production amount in tons range.

For this challenging task, new technologies are now available: high throughput instrumentation, chemometrics, laboratory automation, in line or on line in process analytic have been added to the established high sophisticated technologies such as thermal analysis and calorimetry, X-ray diffraction, spectroscopy and combined techniques. On the other side, predictive computer tools are also available and the two approaches pushed academia and industry to apply better models and better tools in terms of precision and accuracy.

Precise knowledge of thermodynamic stability and relationships between different solid phases is a pre-requisite for the manufacture of robust drug substance and drug products. It is also necessary to know the equilibration curves between the solid forms under the influence of the parameters humidity, temperature and pressure in order to predict changes for storage, stability, compatibility and pharmaceutical processes. The major hurdle for the pharmaceutical industry is to have to recall medicines because of polymorphism problems as it was the case for Ritonavir [13].

Strategic development for both salt form and polymorphism are also reviewed in references [14–22].

Adequate very sensitive quantification methods are needed for the development and are also now required for the monitoring of undesirable solid form(s).

Polymorphic screening

The step one of the ICH guideline is the polymorphism screening. Since polymorphism is to be considered for each salt candidate, it is not possible to separate salt selection and polymorphism selection [17, 18].

Different solid phases can be stable in different environments. For example in case of enantiotropy, at temperature below the transition point, the low melting form is the thermodynamic stable form. A hydrate can crystallize from aqueous solution and an anhydrous phase from organic solutions, both forms being stable in the context of crystallization. An anhydrous phase can be stable at low relative humidity (RH) and a hydrate at high relative humidity. But 'metastable' or 'unstable' forms may coexist resulting from supersaturation or from desolvation or dehydration. All possibilities have to be explored in a polymorphism screening.

In organic chemistry, metastable forms may survive years if a considerable activation energy barrier has to be overcome in moving from the metastable state to the stable state. This activation-energy barrier may be reduced by moisture, catalysts, impurities, excipients, temperature and the transformation into the stable form occurs spontaneously. Seeds of the stable form may accelerate transformations.

In the last step of synthesis, the temperature is generally lowered during crystallization. At the beginning of the crystallization the metastable form which is more soluble, may crystallize first, depending on the metastable zone of crystallization. Through solvent mediated transition the metastable form should be transformed into the stable form. But depending of the crystal growth and of the solubility, this transformation may not happen and the metastable form remains in the solid phase. In the case of hydrates, the water activity of the solvent or of the atmosphere is the key parameter for hydrate formation. The formation of solvates and hydrates is often the source of metastable anhydrous forms obtained during drying.

The number of solid phases which may be correlated with a drug substance can be very high if one considers that a drug substance may form several solvates with each solvent.

Many examples show that in early development metastable forms or amorphous solids are first obtained and that during the development the unexpected thermodynamic stable form appears (6, 30–31). Figure 1 emphasizes the relevance of the discovery of a new highly insoluble form in a liquid formulation. This new form growths slowly from a liquid formulation (Fig.1a). The new form was very insoluble and it was necessary to develop a new formulation in which the new form could be dissolved in the concentration needed for the therapy. The solubility of both crystalline forms were measured in a great number of liquid systems at different temperatures. Figure 1b illustrates the observed behavior of the solubility results, due to kinetic transformation of the metastable form into the insoluble form. By increasing the temperature, the 'apparent solubility' of the metastable form decreases since the transformation into the stable form is accelerated by the increase of temperature.

All polymorphic screening procedures consider crystallizations and parameters such as temperature, co-precipitation, slurry [23–26] in different solvents and in dif-

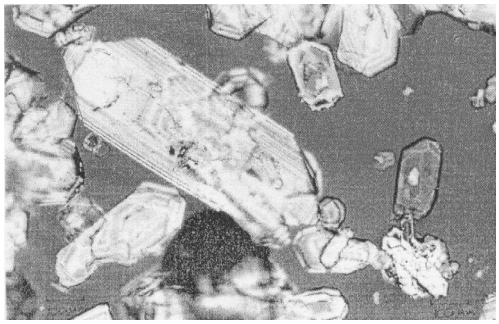

Fig. 1a Slow formation of a new very insoluble crystalline modification during storage of a liquid formulation

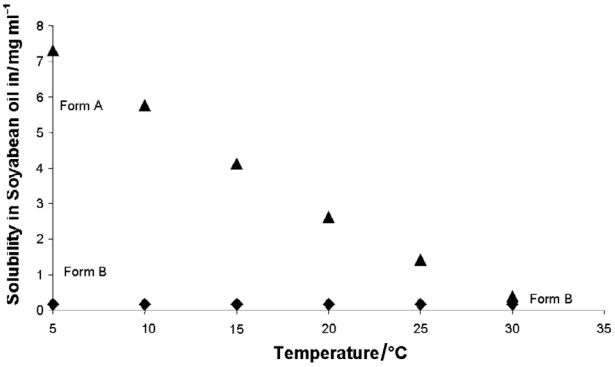

Fig. 1b Comparison of the equilibrium solubility measured with the two forms after equilibration 24 hours at different temperatures. The solvent mediated transformation of the soluble form A into the insoluble form B is accelerated when the temperature of measurement increases

ferent conditions in order to explore the parameters of manufacture. High throughput systems are now considered at drug discovery stage [27–29].

Quenching from the melt, lyophilization, precipitation by adding a co-solvent allow us to obtain the amorphous state.

Heating/cooling curves in DSC or by combined techniques permit to observe forms which are not obtained from solvents [6, 32–36].

The strategy of the screening should permit to:

- Obtain the drug candidate as crystalline form (via salts if feasible)
- Detect solvate formation
- Manufacture 'unstable' or 'metastable' forms
- Study hydrate formation by water sorption-desorption experiments (Hygroscopicity)
- Consider solvate formation by adding solvent atmosphere studies to the crystallizations
- Manufacture amorphous phase

Table 1 Methods for the study of polymorphism

Method	Data measured	Feature
Differential scanning calorimetry (DSC)	Heat flow *vs.* temperature	Fast, very sensitive, automation Best thermodynamic informations Study glass transition Heating, cooling, tempering in situ Combined techniques, X-ray, IR, TG Quantitation limited due to kinetic effects Impurities modify melting points
Microcalorimetry	Heat flow *vs.* time	Quantitation of amorph
Solution calorimetry	Heat flow during dissolution	Quantitation of polymorphs and amorph Generally low energy differences
Thermogravimetry (TG)	Change of mass *vs.* temperature	Fast, very sensitive, automation Study solvates, hydrates Combined MS, IR Quantitation Release and stability testing
Moisture sorption/desorption isotherms	Change of mass *vs.* variable RH%	Hygroscopicity behaviour. Prediction for storage, handling Hydrate formation, crystallization of amorph Several cycles (kinetic of hydrate formation)
FT-IR, DRIFT, ATR	IR spectrum	Chemical information Combined with heat cell, microscopy Information for solvent and solvates Sample preparation artefacts possible Influence humidity during preparation Quantitation possible. LOD substance specific

Table 1 Continued

Raman	Raman spectrum	Complementary information to IR Combined with microscopy No sample preparation Quantitation possible. LOD substance specific
Solid-state NMR	Magnetic resonance	Chemical information Phase characterization Quantitation
X-ray diffraction	Diffractogram	Correlation with crystal structure Crystal structure calculation from X-ray powder data X-ray pattern of metastable forms in situ Heating, cooling, chambers with variable RH% X-ray pattern purity by comparison Mostly used for quantitation. LOD may attain 0.5% Cristallinity measurement Influence particle size and orientation Influence humidity during measurement
Solubility	Amount dissolved in different solvents or temperatures	Characteristic data needed Influence of change during measurement Influence impurities Solubility *vs.* temperature => transition point Saturation solubility => analysis of insoluble Solvent mediated transition => stable form
Microscopy, SEM	Microscopy under the influence of light or electron radiation	Morphology, Surface examination Thermomicroscopy, heating, cooling IR-microscopy, Raman-microscopy

- Obtain thermodynamic stable form(s) in crystallisation system(s) depending on temperature, pH.., in atmosphere depending on temperature and RH%, in drug product
- Consider effect of pressure and milling or grinding

The analytical method for the detection is generally X-ray diffraction with subsequent analysis by DSC and TG. Raman is increasingly used. Each method has its advantages and its limitations as summarized in the Table 1.

The major issue of this step is the proper interpretation of findings:

- The solvate formation is not observed if drying of the solid phases occurs before analysis
- Hydrate formation takes place if study performed at water activity above the critical water activity of hydrate formation
- A phase transformation may occur before or during analysis
- There may be formation of degradation product with the solvent
- Degradation of the drug substance at the temperature of drying may occur
- The dissociation of the salt form or counter-ion exchange in buffer medium has to be taken into consideration
- Impurities inhibit the formation of the stable form
- Mixtures of forms may not be recognised as mixtures

Once the screening is performed, results have to be interpreted and solid phases manufactured as pure phases in order to be characterised according to the ICH step 2.

Interpretation, thermodynamic relationships, selection of the solid form

Thermodynamic basis

The interpretation of the results of polymorphic screen as well as the design of the study are based on the thermodynamic following basis.

The relationships between different phases are governed by the Gibbs phase rule

$$V = C + 2 - \Psi \quad (1)$$

V = variance, C = number of constituants, Ψ = number of phases.

In the case of polymorphism C=1. If two solid phases are present and if both pressure and temperature vary, the variance is one. If the pressure is fixed, the variance is zero. Phase diagrams of pressure *vs*. temperature illustrate the different equilibrium curves for polymorphism.

For each solid form, there is a solid-liquid equilibrium curve and a solid-vapour equilibrium curve. In case of 'enantiotropy', there is a solid 1 <-> solid 2 equilibrium curve and a reversible transition point 1<->2 at a specific pressure. At the transition point, the free energy of the two forms is the same. In case of 'monotropy' there is no thermodynamic transition between two phases since only one solid form is thermodynamically stable.

In the case of enantiotropy, the low melting form is the thermodynamic stable form below the transition point and above this point the high melting form is the thermodynamic stable form. The transition point can be low, close to 40°C in the case of tolbutamide [7, 32] or close to 100°C in the case of propylphenazone or even higher than 200°C [6].

In the case of monotropy there is only the high melting form which is the thermodynamic stable form within the whole temperature range. Figures 2 and 3 illustrate the DSC behaviour with a solid-solid transition before the melting point in case of enantiotropy and monotropy respectively.

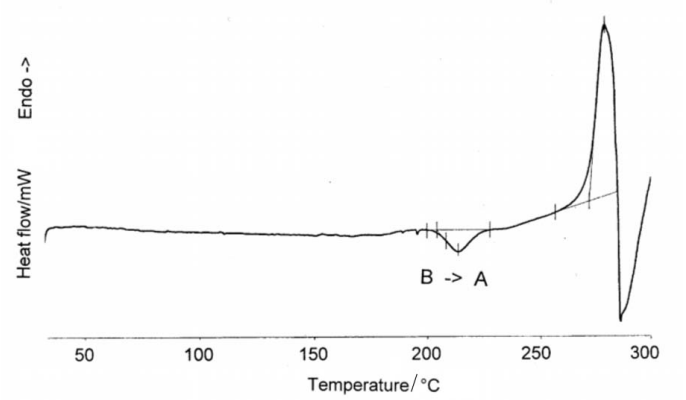

Fig. 2 DSC curve of a metastable form which transforms into the stable form with exotherm (monotropy)

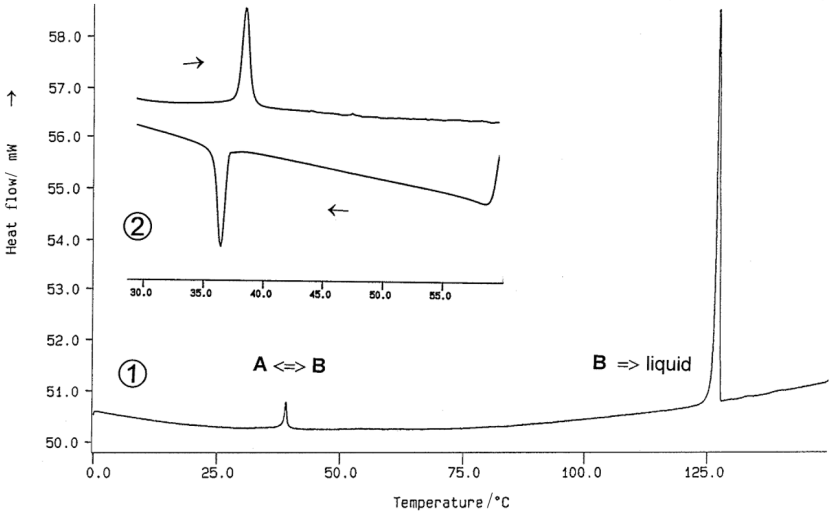

Fig. 3 DSC curve of tolbutamide with a completely reversible enantiotropic transition

The energy diagrams *G*, *H vs.* temperature at a given pressure reflect the transition observed between solid phases, and between solid and liquid phases. At the thermodynamic transition temperature both phases have the same free energy *G*.

The relationship between melting enthalpies of two solid phases *A* and *B* and the heat of transition is approximately

$$\Delta H_t = \Delta H^f_A - \Delta H^f_B \quad (2)$$

According to Burger'rule [37], in the case of enantiotropy, the lower melting form has the higher melting enthalpy and the transition into the high melting form by heating is endothermic. In the case of monotropy, the thermodynamic stable form is the higher melting form with the higher melting enthalpy. The transformation of the 'unstable' form into the stable form is exothermic. A detailed discussion of phase diagrams has been presented by Toscani [38] and by Yu [39].

Differential scanning calorimetry (DSC) which measures every heat flow changes upon heating or cooling is the most appropriate technique since the method allows us to determine the melting points, the melting enthalpies as well as the transition points and the corresponding energy. The influence of kinetic can be followed by using different heating rates and tempering in situ [6].

The phase diagrams of solvates and hydrates are more complex since binary mixtures are implied with different compositions. The new compound may have a congruent melting or a non-congruent melting. By heating, the melting of the hydrate

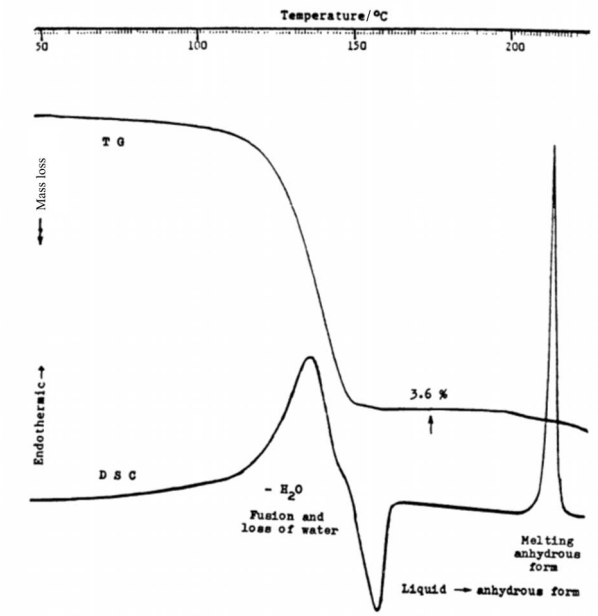

Fig. 4 DSC and TG of a monohydrate: melting and recrystalllisation from the melt with loss of water into the anhydrous form

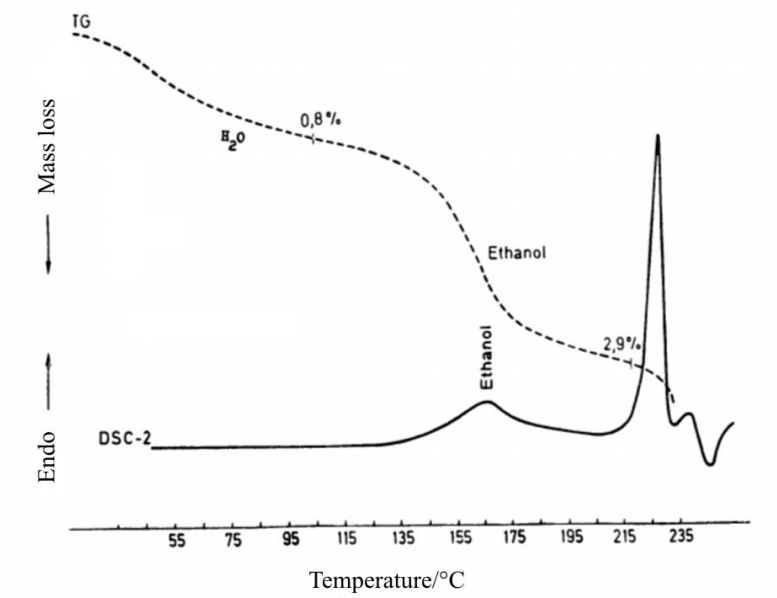

Fig. 5 DSC and TG of an ethanol solvate. Endothermic desolvatation at high temperature

may be observed followed by transformation to an anhydrous form, or the solvent is evolved with an endothermic transition into the anhydrous form (Figs 4 and 5). A serie of such binary phase diagrams have to be considered if several compounds are formed. These diagrams are fundamental for the understanding of crystallisation and drying steps. In heating experiments, tight sealed pan are preferred if one wishes to observe a melting point of a solvate. But for comparison with other techniques, open cells may be advantageous. Investigation of solvates are not possible without thermogravimetry.

If a physical property of a crystalline substance is plotted *vs.* temperature, a sharp discontinuity occurs at the melting point. For amorphous substances, there is no melting point, and a change of the baseline of the DSC curve occurs at the so-called glass transition temperature T_g. Below this temperature, the amorphous phase has certain properties of a crystalline solid (e.g. plastic deformation) and is termed 'glassy'. Above this temperature, the substance retains some of the properties of a liquid, e.g., molecular mobility, and is termed 'rubbery'. Above this temperature, the increase in molecular mobility facilitates spontaneous crystallization into the crystalline form with an exothermic enthalpy change after the glass transition (DSC, Fig. 6). The glass transition temperature, T_g, is lowered by water or other additives such as solvents, facilitating crystallization. The amorphous state is unstable and the study of the glass transition with excipients under humidity is part of the preformulation. Amorphous solid phases are obtained either by quenching from the melt if no degradation occurs during the melting or by lyophilization. They are easily

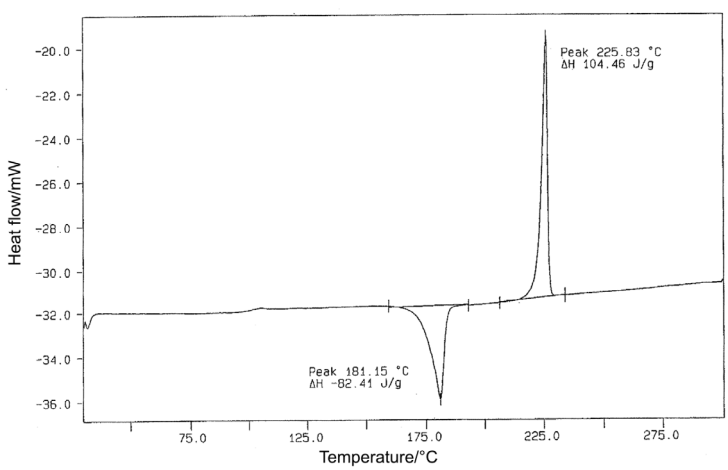

Fig. 6 Typical DSC curve of an amorphous drug substance. Glass transition at approx. 100°C followed by exothermic crystallization followed by the melting of the crystalline form

detected by DSC – where the glass transition can be followed by the exothermic crystallization into the crystalline state – and by X-ray diffraction.

Kinetic factors are responsible for the existence of solid phases outside thermodynamic phase diagrams [8, 40–41]. Figure 7 illustrates the influence of the addition

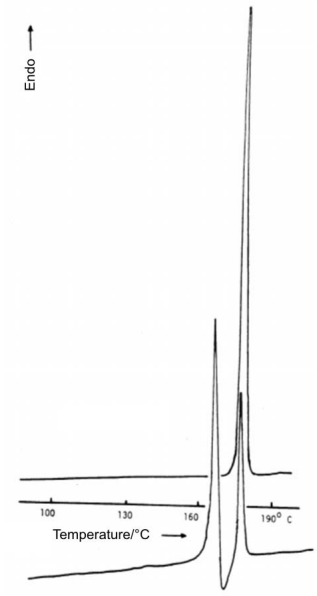

Fig. 7 Metastable form of a drug substance obtained if 2% of impurity is spiked in solution before evaporation. Top: stable form, bottom: metastable form (monotropy)

of 2% of a by-product of proquazone in the crystallization experiment in ethanol for the detection of a metastable form, not obtained with the current quality [42]. Figure 8 illustrates the influence of seeds of a stable form on the transformation of a batch into the less soluble form while the other batch which is free of the stable form, remains unchanged [40, 41]. A DSC kinetic method based on Avrami model has been proposed to determine the amount of seeds in batches [43]. The influence of impurities and kinetics are discussed in [6, 44].

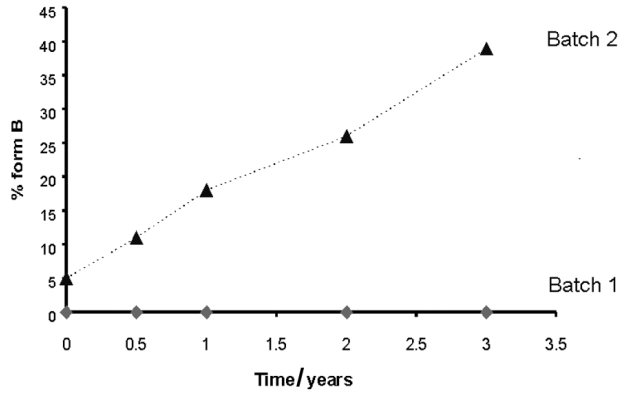

Fig. 8 Content of the stable form B in samples of two batches of the metastable form A stored in tropical climate measured by X-ray diffraction. Seeds of form B initiate the transformation in batch 2 while no change is observed in batch 1

The measurement of solubility at different temperatures needs more experiments but gives also relation to the thermodynamic relationships. This is specially recommended for a robust process in the solvent of crystallization (6, 23–26). For each modification

$$\text{Log } C = -\Delta H_{\text{dissol}} / RT + K \text{ is valid.} \quad (3)$$

where C is the solubility, T the temperature in Kelvin, R the gas constant and ΔH_{dissol} is the heat of dissolution (or heat of solution) in a solvent and K is a constant. In case of enantiotropy, both forms have the same solubility at the temperature of transition, in case of solvates or hydrates, there is also the same solubilty at the point of transition, e.g. monohydrate-anhydrous. In the case of monotropy there is no transition point. The less soluble form is the stable form regardless of the temperature.

Solution calorimetry allows to determine the value of ΔH_{dissol} of each form, the difference gives also the heat of transition ΔH_{t}. The method is useful in those cases where the melting occurs with decompositon, but without a rough estimation of the melting point, the results do not help to distinguish between enantiotropy or monotropy.

Transformations to the equilibrium, that means to the stable form, may be accelerated by the presence of a solvent (3, 6). *Solvent mediated transformations* occur by a continuous dissolution-crystallization process. This property can be used successfully in addition to the Burger's rule to establish thermodynamic relationships. *By*

studying the solid phase of slurries at different temperatures, one can expect to find the domain of stability of each solid phase. By mixing polymorphs, the transformation is accelerated as demonstrated for MKS492 in Fig. 9 [34]. However it might be that a solvent or an additive inhibits the transformation or that a solvate formation might lead to false interpretation [45].

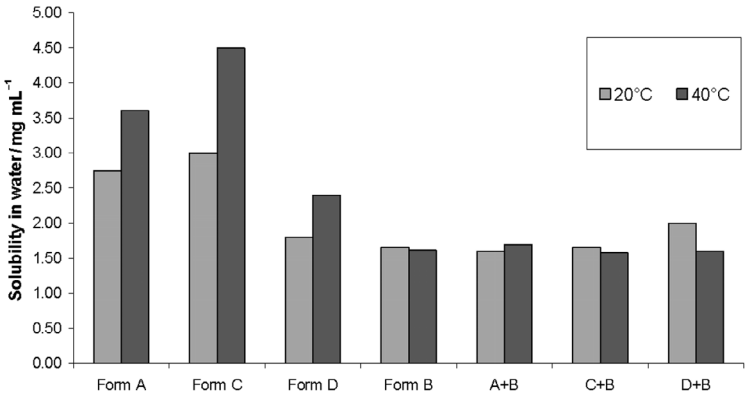

Fig. 9 Solubility of 4 forms of MKS492 in water at 20°C and 40°C. A, C and D are monotrops to B. If B is added, the solvent mediated transformation occurs immediately

Isothermal microcalorimetry has been proposed to follow phase transitions of seradostrat [46].

Solvates and hydrates are more complex. Detailed reviews are given in [47, 48]. The influence of the pressure on the phase diagram has been discussed by Soustelle [49]. Pressure DSC in the study of hydrates has been successfully applied [50–51]. Polymorphism is also often observed for hydrates [52–54]. The eutectic behavior of hydrates has to be taken into consideration for the interpretation of thermal analysis data [55]. Sub-ambient DSC has been proposed for the determination of bound water in the study of crystalline hydrates [56]. The critical water activity of the solvent of crystallization is highly relevant for the formation of hydrates and small amount of water in the industrial solvent or in the atmosphere during drying are sufficient to induce the formation of hydrate [57–59].

Interpretation

Thermal analysis combined with heating X-ray diffraction or IR and TG-MS allow preventing wrong interpretation. A new X-ray diffraction pattern as well as a new DSC peak is not sufficient to conclude to a new polymorph. In one typical case, we could demonstrate the degradation of a malonate during melting into the corresponding base [36]. We observed degradation product with the solvent or during the drying. In this last case, IR gave concluding interpretation as well as the HPLC analysis

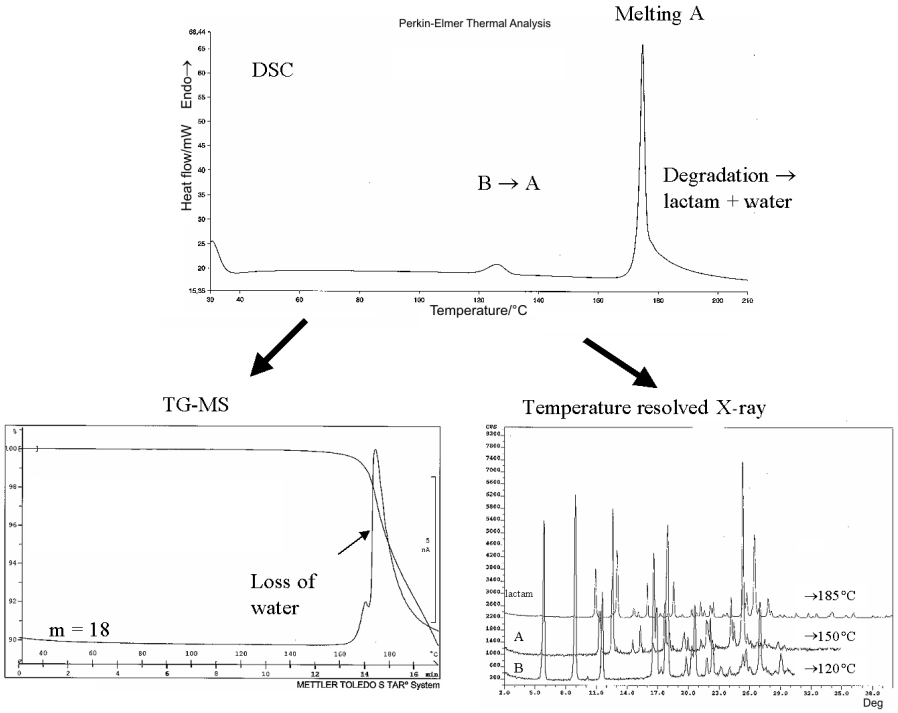

Fig. 10a Combined techniques for the study of a drug substance with enantiotropic behaviour and degradation into a lactam. DSC and heating X-ray study show the enantiotropic transition at 150 °C and the formation of a new phase at 185°C with the melting. The TG-MS shows the loss of water during the melting

[16, 60, 61]. Figure 10 shows the DSC curve, the corresponding heating X-ray study, the IR heating study and the TG-MS. The sample undergoes a reversible solid-state transition A->B followed by melting and degradation: water elimination. The DSC as well as the heating X-ray diffraction confirm the enantiotropic relation between two forms and the degradation during melting into the lactam is demonstrated by heating-IR and TG-MS. Typically observed for salts is the dissociation into the base and they might be different polymorphs of the base [16].

Selection of the solid form, case of hydrates and labile solvates

The selection of the candidate can be difficult in case of dehydration, hydration or solvate formation.

The following old example of a drug in development emphasises the advantage of an on-line monitoring which are today available.

The solvent chosen for crystallisation was ethanol, but the observed behaviour did not comply with the polymorphic studies. By crystallisation and slurry experiments in different solvents, two solvent-free forms A and B as well as stable solvates (ratio 1 :1)

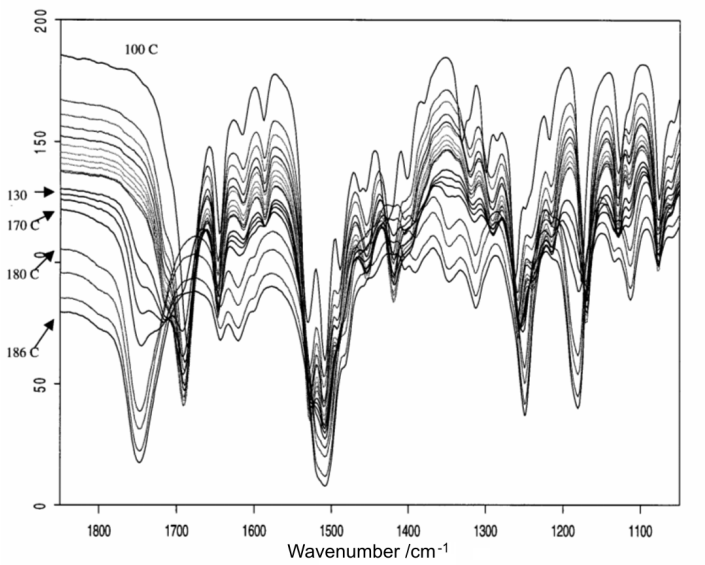

Fig. 10b Combined techniques for the study of a drug substance with enantiotropic behaviour and degradation into a lactam. Heating FT-IR experiment: After polymorphic change at 120°C, apparition of a new band corresponding to a new chemical structure assigned to the lactam

with acetone and isopropanol were identified by DSC, TG, IR and X-ray diffraction. Upon drying the solvates transform into the form A. In DSC, the form A and B melt with decomposition. The DSC of the form B show two peaks with a transformation into A within the decomposition process as demonstrated by X-ray diffraction.

In ethanol form A was obtained at temperatures <40°C and B at temperatures >50°C. The hypothesis of a transition A⇔B between 40 and 50°C was not in line with the results of slurry experiments in other solvents for which B was always obtained.

In order to develop a robust crystallisation process, a study in depth was undertaken.

In all slurry experiments by using samples of form A, form B and mixtures thereof, form B is obtained via solvent-mediated transition in methanol, water, acetone and ethylacetate at different temperatures (10, 25, 40, 50, 60°C). At temperatures <50°C, the solvates are formed with isopropanol and acetone and B is obtained at higher temperatures. In mixtures ethanol/water or ethanol/ethylacetate the form B is obtained when a certain amount of co-solvent is added.

The solubility in water was measured *vs.* time and the true solubility extrapolated. Form A had a solubility of 0.16% at 25°C while the form B had a lower solubility of 0.12%. The solubility *vs.* temperature was also measured in ethanol. Form B was less soluble than A if the temperature was >50°C (1.6% compared to 1.8% at 50°C), but at lower temperature A seemed to be less soluble. This was explained by a faster transformation of form A into an ethanol solvate that had a structure very similar to the form A. The solvent of crystallisation ethanol/ethylacetate was chosen for a

robust process with the obtention of the form B whatever the temperature of crystallisation. A quantitative X-ray diffraction method was developed for release and stability analysis [63] .

Are the solid phases obtained pure or mixtures?

If a mixture is always obtained in the polymorphic screening, new findings may occur during manufacturing up-scale and the starting of development can be wrong. Typical cases are solvates with very similar X-ray pattern. TG and TG-MS or GC or NMR are necessary.

Following case exemplifies the advantage of the kwnowledge of crystal structure of the polymorphs: ultimate demonstration of the phase purity of samples.

The drug substance was a malonate candidate. The first sample studied was the first laboratory batch. Crystallization and slurry experiments in different solvents and at different temperatures did not show relevant changes by X-ray diffraction nor by DSC. Some experiments in slurries showed only that one or two peaks in the X-ray diffraction pattern disappeared. In some media, like dimethylformamide, ethanol/water mixtures, peaks of the corresponding base appeared.

It was believed that only one crystalline form was present and experiments were undertaken to determine the single crystal structure. At slow crystallization performed in a saturated DMF solution to growth adequate crystals for single crystal analysis, crystals with different morphologies were observed. The two types of crystals were analysed and the single crystal structure of two pure polymorphs could be determined.

These findings oriented differently the interpretation of the polymorphism program and the crystallization of the manufacturing process. The calculated X-ray diffraction pattern of the single modifications A and B allowed to monitor the development of the crystallization process. Conditions were found to obtain pure A or pure B.

The DSC behaviour of the both forms were very similar: melting at approx. 190°C followed by decomposition so that the energy Burger's rule could not be used as an indication of thermodynamic relationship. Heat of dissolution are identical. Dichloromethane was found the most appropriate solvent to follow solvent mediated transitions. In slurry experiments of mixtures A+B, A was obtained at 20°C and B at 50°C. The solubility measurements at different temperatures of both forms in methanol suggested that the less soluble form A transformed into B at 40–50°C and that the thermodynamic relationship should be enantiotropic between both forms. The anhydrous modification A is monoclinic with the space group $P2_1$ and the anhydrous modification B is orthorhombic with the space group $P2_12_12_1$. Both forms have a very similar conformation and modelling study explains this behaviour [62].

Place of X-ray diffraction, crystal modeling

The crystal structure of polymorphs is the best evidence of the composition of solid phases. Some substances have the property to give solvates with almost all solvents with very similar powder patterns. The crystal structure obtained by single crystal

diffraction or by computational calculation from X-ray powder diffraction helps considerably to understand the transition in the solid-state: cohesive desolvation with formation of metastable desolvated phase or disruptive desolvation. These new possibilities of the use of X-ray diffraction open deep insights in the understanding of the polymorphic behavior of new active ingredients [64–66]. Price [67] recently reviews the progress of polymorphism prediction, which unfortunately does not allow yet to predict accurately polymorphs and their thermodynamic relationships.

The advantage of crystal modeling is unique for its ability to demonstrate purity of solid phases, but has limits in case of solvates where thermal analysis and GC analysis take a great place.

Relationship between polymorphs, examples

DSC, TG combined with X-ray diffraction, Solvent mediated studies, water sorption-desorption and sub-ambient DSC for the determination of bound water are necessary for the study in depth of thermodynamic relationship between solid phases of a drug candidate as shown for tetracaine hydrochloride in Fig. 11 [32].

In case of solvates the use of TG-MS is additionally very efficient as demonstrated in Fig. 12. It was possible in laboratory scale to obtain form A in acetone as first solvent of crystallization with appropriate temperature of crystallization and drying conditions. Upscale was not successful. The study in acetone, ethanol, methanol and tetrahydrofurane showed the formation of solvates which upon heating transform into an intermediate form B' which transform into A by further heating or into B

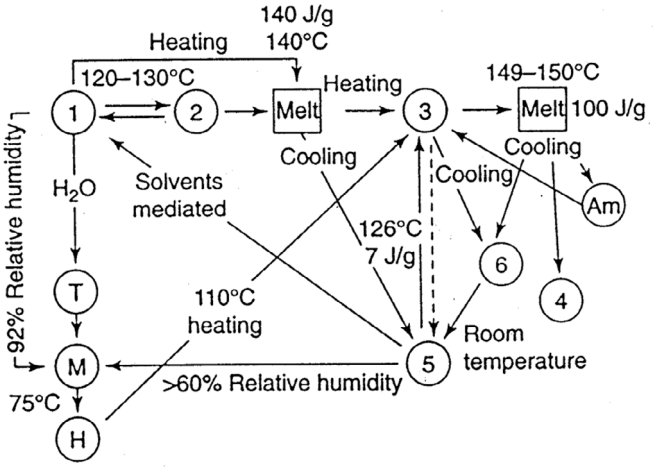

Fig. 11 Relationships between the solid phases of tetracaine hydrochloride·6 crystalline anhydrous forms were identified, an amorphous form, a tetrahydrate, a monohydrate and a hemi-hydrate. At room temperature only the anhydrous forms 1 and 5 are stable. The tetrahydrate is only obtained by crystallization from water. The monohydrate is obtained by storage at 92%RH. By heating, the hemi-hydrate is obtained at 75°C and transforms into the high melting form 3 which is stable only at high temperature

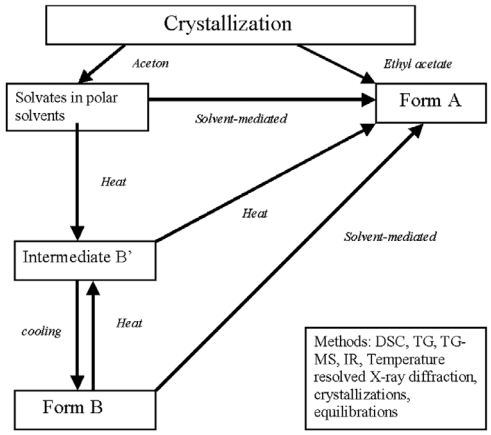

Fig. 12 Relationships between solvates, intermediate form B' and the metastable form B obtained at room temperature and the stable form A. B and B' are monotrops to A

by cooling. Temperature resolved X-ray diffraction and DSC and TG-MS are given in Figs 13 and 14. All solvates and form B transform into A by solvent mediated transition in water and ethyl actetate [60, 61].

The third example in Fig. 15 deals with a sodium salt. Solvates, hydrates, metastable forms as well as different salt forms have to be considered for a robust process [16]. In this case, most of the solid phases were confirmed by single crystal structure determination.

Characterisation

All physico-chemical characteristics of the solid-state are involved in the polymorphism and pseudo-polymorphism. The main properties affected are morphology (crystal shape), volume, density, melting and sublimation temperatures, heat capacity, conductivity, viscosity, crystal hardness, crystal shape, color, refractive index, solubility, dissolution rate, stability, hygroscopicity, processability and solid-state reactions.

Morphology

The morphology of crystal is not obligatory correlated with polymorphism. However, the crystal structure indicates the preferred morphology as demonstrated with scanning electron microscopy in Fig. 16. There is a good correlation between the predicted morphology and the observed morphology.

Density

It is measured by pycnometry and is also a parameter given by the single X-ray crystal structure. According to the polymorphism rule of Burger, the higher the density, the higher the stability. But there are some exceptions.

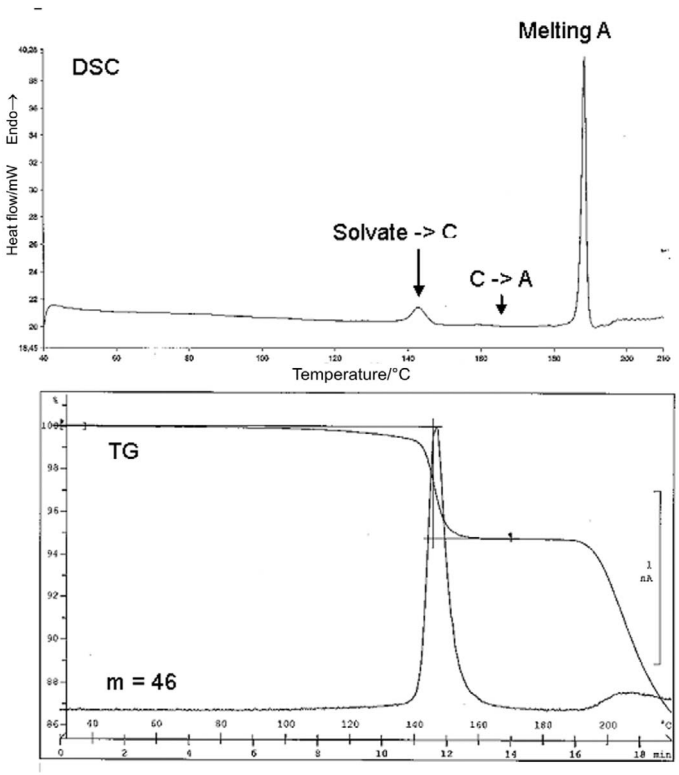

Fig.13 DSC and TG-MS curves of the ethanol solvate corresponding to Fig. 12

Melting point

The melting point of organic substances is one of the first properties measured. A number of techniques are available, from immediate melting to the capillary method described in various pharmacopoeias. The substance is heated and the transition to the liquid phase is observed visually or by hot-stage microscopy. Generally the melting point is measured by DSC. Some polymorphs may have differences of melting points less than 1°C or differences more than 100°C [6].

Solubility

The impact of solubility to polymorphism has to be taken into account. Dissolved concentrations should be measured as a function of time when determining the solubility of the different forms because of possible transformation during the measurement. Furthermore, for proper interpretation of the results of solubility measurements, the temperature has to be maintained constantly and accurately defined.

Solubility can be measured by phase solubility analysis, where an excess of the solid remains in equilibrium with the saturated solution. The solution is analysed by

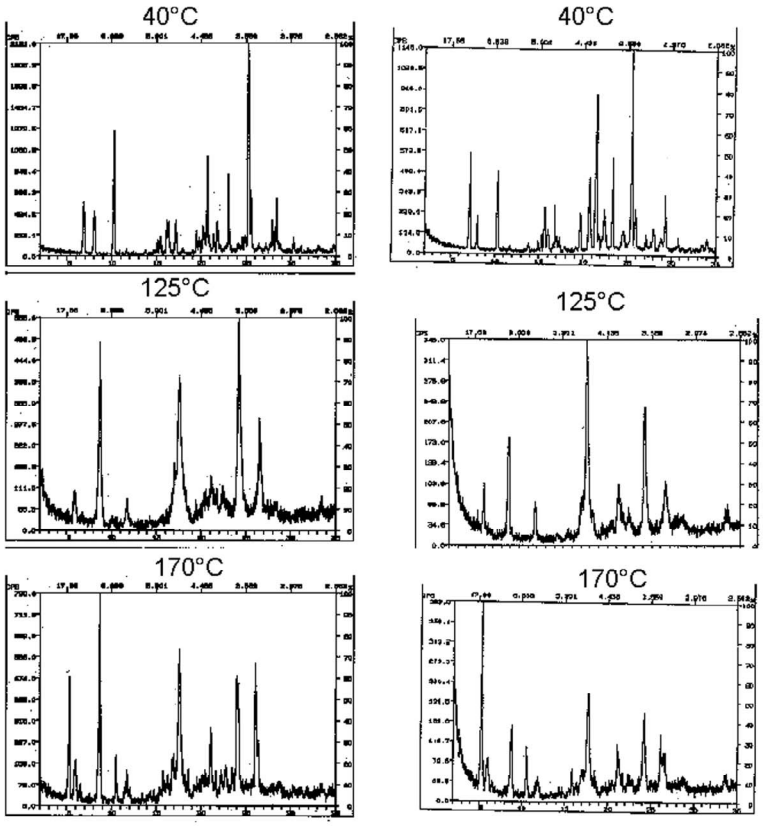

Fig. 14 Diffractograms obtained with heating X-ray diffraction of the acetone (left) and the ethanol (right) solvates

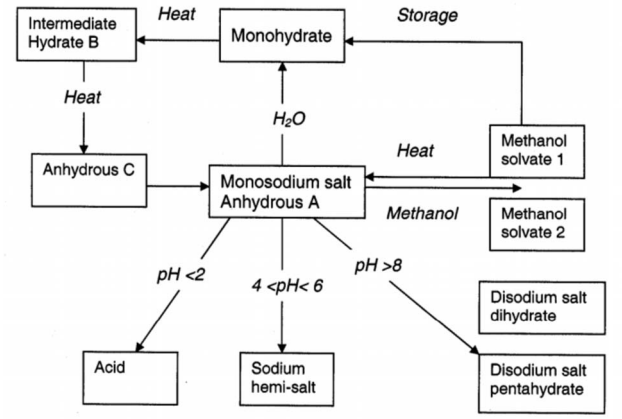

Fig. 15 Example of solid phases to consider for a monosodium salt of a drug substance

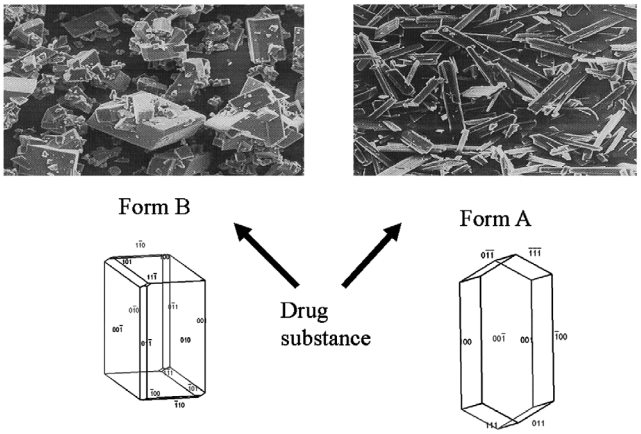

Fig. 16 Comparison of the calculated morphology from single crystal structure with the experimental morphology for two polymorphs of a drug substance

gravimetry, high performance liquid chromatography (HPLC), or spectroscopy. High throughput methods of solubility determination have been developed based on the measurement of the appearance of the solid phase by nephelometry or by ultraviolet spectrophotometry. In all solubility determinations, soluble impurities can significantly influence the measured solubility.

The equilibrium solubility must be always carried out with the knowledge of the different phases and with the analysis of the residual solid. Several thermodynamic stable species may exist depending on the solvent, the pH, the temperature, the relative humidity, it is more appropriate to define the 'equilibrium solubility' of the stable forms in the medium and at the temperature considered. Table 2 is an example of 'equilibrium solubility' for which the solubility is determined for the trihydrate, the monohydrate and the anhydrous form of the drug [54]. In case of salts, the exchange of the counter-ion with the buffers has to be considered [17].

Table 2 Equlibrium solubility in water of the trihydrate, the monohydrate and the anhydrate of a drug substance

Temperature /°C	Solubility /mg ml^{-1}	Residual solid
10	2.1	trihydrate
25	2.8	trihydrate
40	10.9	trihydrate
60	25.3	monohydrate
80	31.6	anhydrate

Dissolution rate, Intrinsic Dissolution Rate (IDR)

The dissolution rate depends on the particle size [4]. The dissolution rates of powders are often measured by the flow-cell method [68]. Figure 17 deals with a drug substance

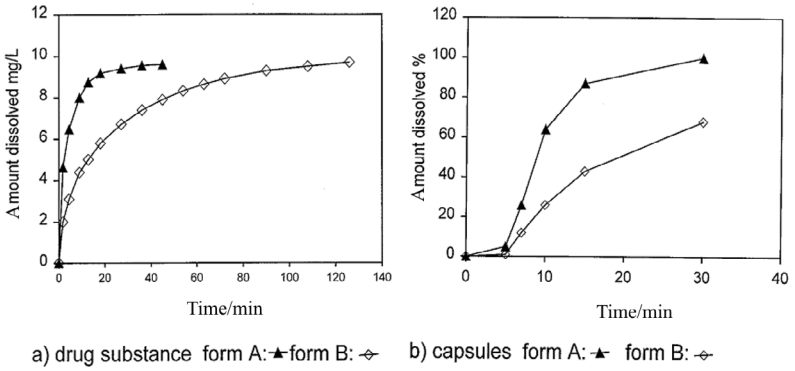

Fig. 17 Dissolution rate curves of two crystalline modifications (A and B) of a drug candidate with the same particle size distribution and the corresponding curves of the drug products consisting of capsules containing A and B

with polymorphic behaviour [6, 18]. The dissolution rate of two samples of the drug substance, representing two polymorphs of almost the same particle size, is reflected in the dissolution rate of the drug product as capsules. Polymorphic transformations as well as hydrate formation and dissociation of salts may occur resulting in change of the slope of the curves and the dissolution of metastable forms cannot be measured [69].

The dissolution rate per unit surface area, termed the intrinsic dissolution rate is independent of the particle size and is therefore very appropriate for polymorphic studies. In the 'disc' method, the powder is compressed by a punch in a die to produce a compact disc or tablet. The method is described in USP [70]. Only one face of the disc is exposed to the dissolution medium and the cumulative amount dissolved per unit surface area is determined by ultraviolet spectrophotometry until 10% of the solid is dissolved. The slope of the plot of mass dissolved per unit surface area *vs.* time gives the intrinsic dissolution rate in appropriate units, e.g., mg min^{-1} cm^{-2}. If a change in the slope is observed during the course of the experiment, then a change in the solid phase exposed to the solvent is occurring during the experiment. Dissociation of the unionized form as well as a polymorphic change can occur [17, 18].

The ratio of intrinsic dissolution of two phases is the same ratio as their solubility.

Polymorphism has its relevance as demonstrated in Table 3 for poorly soluble substances. The amorphous form improves the behaviour, but the factor is <5.

Table 3 Impact of polymorphism on the Intrinsic Dissolution Rate (IDR)

Drug substance as base		Drug substance neutral	
Polymorph	IDR in water with 0.2% LDAO	Polymorph	IDR in water/ mg min^{-1} cm^{-2}
Amorphous form	0.048	Amorphous form	0.269
Form B	0.035	Form A	0.117
Form D	0.011	Form B	0.085

Table 4 Impact of salt and of hydrate formation on the Intrinsic Dissolution Rate (IDR)

Salt form	IDR/ mg min^{-1} cm^{-2} water	IDR/ mg min^{-1} cm^{-2} buffer pH 6.8
Monosalt Na	43.6	22.6
Monosalt monohydrate	17.6	16.5
Hemisalt	0.40	0.35

If different salt forms with the same counter ion are possible, their IDR is also very relevant as demonstrated in Table 4. The monosodium salt is very soluble. The monohydrate is less soluble, but the IDR of the hemi-salt decreases by a factor of approx. 100.

Heat of solution

The enthalpy of the solution, i.e., heat of solution or dissolution, can be calculated from the temperature dependence of the solubility. This method has been widely used. However, Hollenbeck [71] and Burger [72] consider that the slope of the linear relation predicted by solubility data is too variable, and is subject to systematic errors of several percent due to a non-ideal saturated solution, resulting in an inaccurate determination of both the heat of solution and the transition point of two forms. The heats of solution of substances can be measured directly by solution calorimetry, which provides more meaningful values. The difference between the heats of solution of the two polymorphs is equal to the transition enthalpy of the polymorphs at the temperature of measurement. The results provide an alternative to DSC for the discrimination between enantiotropy and monotropy, when the substance decomposes upon melting. Quantitative analysis of polymorphs, solvates and amorphous forms in mixtures has been performed using solution calorimetry. Studies of polymorphism by solution calorimetry have been reviewed [6].

Interaction with water vapour expressed by sorption isotherms

Water vapour is an omnipresent component of the atmosphere. The vapour pressure of water at different temperatures has been tabulated. At a given temperature, the ratio, actual water vapour pressure/saturated water vapour pressure at that temperature is termed the *relative humidity* (RH). The natural RH depends on the climatic zone and may vary from 30% to more than 75%.

Limiting the sorption of water vapour from ambient air is crucial for maintaining the quality of some pharmaceuticals during their manufacture. The behaviour of drug substances at different temperatures and humidities for different climates is generally studied by gravimetry. The RH for gravimetric studies of water sorption and desorption can be controlled by saturated salts solutions [73] or by continuous humidification of a stream of air or nitrogen to which the solid sample is exposed. In the dynamic water sorption system [74], the sample is placed on a microbalance, which is exposed to a continu-

ous flow of air or nitrogen with a constant, predetermined RH. Solid-state hydration may occur leading to the formation of hydrates. X-ray diffractometers may be equipped with special sample cells for exposing the sample to controlled temperature and humidity. The structural changes in the solid can be monitored and the extent of their reversibility can be studied. Figure 18 shows the sorption isotherms of two crystalline modifications of a drug substance both of which transform into a hydrated form at 25°C [54]. Under ambient conditions the thermodynamically stable form is less hygroscopic. The critical RH at which the mass changes abruptly, corresponding to the RH at which the formation of the hydrate begins, depends upon the temperature. The higher the temperature the lower is the critical RH. For such behaviour the selection of the form to be developed is a strategic choice. The formation of theophylline monohydrate at the surface of tablets containing anhydrous theophylline is an example that has been thoroughly studied [76]. In the case given on Fig. 18, the selection of the monohydrate was envisaged, but the crystallization experiments revealed the formation of a second polymorphic form of the hydrate and the process was easier and robust with the manufacture of the stable anhydrous polymorph although some handling was necessary to protect it from moisture.

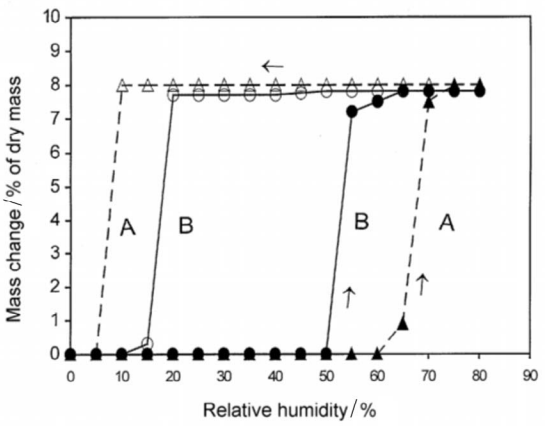

Fig. 18 Examples of water sorption-desorption isotherms of two enantiotrops polymorphs at 25 °C. The two polymorphs, A and B, transform into the same hydrated form. The form B metastable at ambient temperature takes up water at lower RH than the stable form A. The hydrate form loses water at RH values below 20%

Different polymorphs usually behave differently [6]. A metastable polymorph may be transformed into a crystalline hydrate, whereas the stable polymorph may not. Solvates may transform directly into hydrates [75, 77]. When several hydrates are formed, the kinetics of the dehydration processes may be different from the kinetics of the hydration step, as in the case of nafragel hydrochloride [78] for which the sorption of water occurs in two steps via the hemihydrate. The resulting monohydrate loses water in one step yielding the anhydrate. Due to the different kinetics of their transformations, the anhydrate, hemihydrate and monohydrate can coexist for an appreciable period of time.

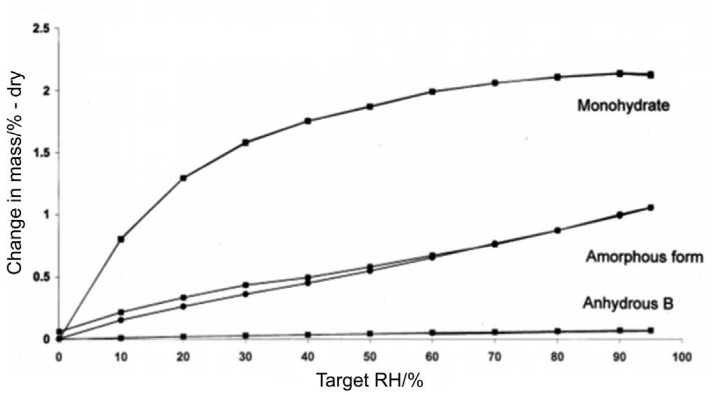

Fig. 19 Sorption-desorption of the anhydrous stable form, the amorphous form and of the reversible hydrate anhydrous metastable forms

Figure 19 shows the hygroscopicity behaviour of the amorphous form, of the anhydrous stable form and of the metastable anhydrous form which transforms reversibly into the monohydrate. The transformation was followed by X-ray diffraction in a cell with variable humidity and also in a heating cell.

Table 5 summarizes the physicochemical characteristics of 4 forms of MKS492 [17]. Form B' is enantiotropically related to form B.

Stability

Study of the stability behaviour of polymorphic forms is a part of the required characterization. Chemical reactivity in the solid-state is correlated with the nature of the crystalline modifications [40]. The amorphous state is very reactive. Table 6 illustrates the difference of reactivity between the 2 crystalline forms and the amorphous form of a drug candidate. The crystalline form A is quite more stable as B and the amorphous form. Higher differences are observed for the 2 polymorphs of a dihydrate. The example 3 deals with a peptide drug candidate. In the amorphous state, both the base and its hydrochloride were very unstable. However, the base could be obtained as a crystalline material with a substantial gain in stability.

Light testing is a part of preformulation [80]. Examples 2 and 3 of the Table 6 show that the stability under light exposure can be very different. Differences were described for chloroquinone di-phosphate [81]. In the case of a drug substance we observed even different degradation products.

Control of polymorphism is a part of development stability. Figure 8 given above corresponded to the formal stability testing of two batches. The results determined by X-ray diffraction were confirmed by IR [40, 41].

Kinetic has to be considered also for hydration as demonstrated in Fig. 20. In this case, the validated quantitative analysis method of X-ray diffraction gives the same results as thermogravimetry (TG) (TG=5.5% for the monohydrate), therefore

Table 5 Example of characterization of polymorphs for MKS492 according to [34]

Characteristic	Form A	Form B	Form B'	Form C	Form D
DSC onset	111°C	–	128°C	118°C	109°C
Melting enthalpy / J g^{-1}	93	98[1)]	92	89	65
Transition heat / J g^{-1} Temperature	–	6 108– 112°C		–	–
Mass loss by TG	<0.5%	<0.5%	–	<0.5%	2%
Morphology	needles	needles		needles	Plates/needles
TG after 1 day at 92% RH	< 0.5%	< 0.5%	–	< 0.5%	>2%
Density / g cm^{-3}	1.400	1.422		1.411	
Solubility water 20°C % (*w/w*)	0.27	0.17		0.2	0.18
Solubility ethanol % (*w/w*) 25°C	2.4	1.5		2.5	
Solubility isopropanol 25°C % (*w/w*)	1.3	0.9		1.5	
Solubility acetone 25°C % (*w/w*)	7.1	4.3		7.2	
Dissolution rate in water at 37°C time / s for 50% time / s for 80%	 88 219	 109 315		 99 330	 340 972
Intrinsic DR, 37°C mg min^{-1} cm^{-2} in water in HCl 0.1M		 0.12 0.18		 0.13 0.19	
Bands IR / cm^{-1}	3438	3502 3551		3444 3309	3274
X-ray angle 2θ / degree	12.7	7.1		4.9	3.8

the TG results in Fig. 20a demonstrates easily the transformation observed during formal stability studies of different batches in different packagings. In very tight packaging (permeation <0.5 mg day L^{-1}) no transformation occurs. The transformation into the monohydrate depends of the batch studied, of the time of storage and of the packaging. For example after storage in polyethylene bags which are permeable to moisture, no transformation is observed for one batch after 12 months at 30°C/70%RH while 2 to 4% transformation is observed for two other batches. After storage at 40°C/75%RH, the results vary from 2% to 11%. This demonstrates the role of kinetic behavior in the phase transformation. The sorption-desorption study with several cycles shows the influence of nuclei. In the first cycle, the uptake of water takes place above 90%RH. After desorption a second cycle was done. Here the moisture is taken at approx. 40%RH (Fig. 20b).

Table 6 Influence of polymorphism on the stability behavior in solid-state

	Degradation (HPLC)	
Example 1	1 month at 80°C (oxygen/water)	
Crystalline form A	No degradation	
Crystalline form B	0.5–1.5% degradation	
Amorphous form	2.0–3.5% degradation	
Example 2	2 weeks at 50°C	Exposition 1200 klux h^{-1}
Monohydrate A	No degradation	10%
Monohydrate B	12%	23%
Example 3	1 week at 70°C	Exposition 300 klux h^{-1}
Crystalline form	10%	2%
Amorphous form	80%	38%

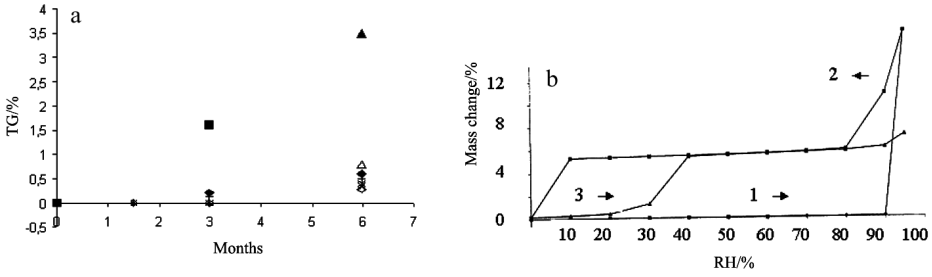

Fig. 20 Formal stability study. Case of a hydrate formation kinetically accelerated by seeds of the hydrate. a – Formation of the monohydrate as followed by TG after long exposition at 40°C/75%RH. b – Sorption-desorption isotherm of the anhydrous form with several cycles. The first cycle of DVS did not show hygroscopicity below 90%RH. After desorption and a second cycle hydration is observed already at low RH of 40%

Quantitative analysis

In general quantitative methods require generally samples of polymorphs for routine analysis, what is often difficult for metastable solid phases. Samples have to be analysed in order to have proper standards.

For quantitative analysis, methods have to be validated with linearity, accuracy, precision, intermediate precision, limit of quantitation and limit of detection.

DSC is very sensitive, however kinetic factors during heating can give erroneous results. For a drug substance we developed a quantitative X-ray diffraction analysis and the LOQ was 2.5%. With DSC, the undesirable form was detected after the melting of the current form. In order to check the absence of the undesirable form which initiates transformation, DSC was adequate. It was easy by DSC to detect less than 0.5% [16].

Papers published by Stephenson *et al*. and Bugay [82, 83] review the efficiency of quantitative X-ray diffraction and spectroscopic methods. The problems of preferred orientation in X-ray diffraction are discussed by Davidovich *et al*. [84] and the simulation by X-ray diffraction is discussed by Yin *et al*. [85]. The selection of one peak is the most used technique, internal standard [86], external standard can be used. Since certain peaks are more sensitive to orientation, it is recommended to check the repeatability of the peak height or the peak area for more precision [41].

Tables 7 and 8 give examples of intermediate precision and accuracy for the quantification of a trihydrate in a monohydrate [16].

Table 7 Example of intermediate precision obtained for the determination of a trihydrate

Relative standard deviation:	29.2%
Number of determinations:	16
Absolute standard deviation:	1.8%
Mean value:	6.3%
Individual values / % analyst 1 analyst 2	 6.67; 4.29; 5.25; 4.84; 12.32; 5.63; 5.58; 5.93 5.37; 5.98; 7.99; 5.49; 6.78; 5.53; 7.81; 5.04

Table 8 Accuracy: Spiking technique of the trihydrate added

Trihydrate added/%	Trihydrate found/%					Average/ %	Absolute standard deviation/ %	Difference to theoretical value/%
	Analysis no.							
	1	2	3	4	5			
1.0	0.78	0.69	0.84			0.8	0.1	0.2
2.1	1.56	1.51	1.60	1.33	1.76	1.6	0.2	0.5
3.1	1.82	1.68	1.54	1.66		1.7	0.1	1.3
5.1	3.22	3.12	3.09	3.29	3.52	3.2	0.2	1.9
8.1	4.71	4.64	5.12	5.47	5.11	5.0	0.3	3.1
10.0	6.28	7.68	6.55			6.9	0.7	3.2
15.0	11.4	10.49	12.02			11.3	0.8	3.7
20.6	18.96	18.44	17.86			18.4	0.6	2.2
30.0	28.34	29.79	28.84			29.0	0.7	1.0

X-ray diffraction has the advantage, that the purity of standards can be checked with the calculated X-ray powder diffraction from crystal structure as demonstrated in Fig. 21. It is also possible to simulate calculations of the limit of detection (LOD). If no pure standard is available, and especially if the standard is partially amorphous, the limit of detection measured is higher as the real situation. We observed a change of LOD from 20 to 10% by using a more crystalline standard.

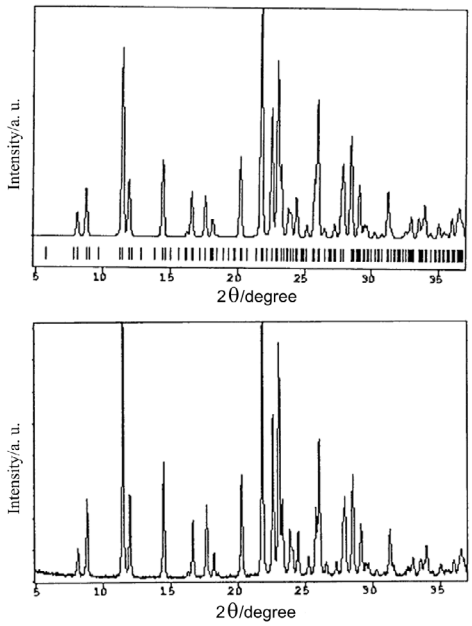

Fig. 21 Comparison of X-ray powder diffraction pattern calculated from single crystal structure (top) and experimental data

Table 9 summarises some examples of the limit of detection obtained for quantitative methods developed by X-ray diffraction in drug substance. Figure 22 shows the use of X-ray diffraction for the quantitative determination of a hydrate in a tablet.

With X-ray diffraction the crystallinity can be also determined by measuring the area of peaks and of the amorphous background. The major difficulty is to be able to find the correct baseline. DSC allows to calculate amorphous content by the calculation of the exothermic recrystallization peak. Table 10 shows that both techniques give comparable results.

Solution calorimetry is a useful technique for the quantitative analysis of polymorphs or of amorphous forms. Table 11 shows the comparison of solution calorimetry, X-ray diffraction and DSC based on the melting enthalpy since no recrystallization occurs by DSC.

IR [87]or best DRIFT or ATR which does not require sample preparation which could produce phase transformation can be used. Comparisons with X-ray diffraction [41, 88, 89] demonstrate that the method is often less sensitive, but in some cases LOD better than 5% could be achieved. NIR is a technique completely accepted by authorities [90] for quantitative analysis by using chemometrics. It has been successfully used for sulfathiazole and indomethacin with LOD in the range of 1% [91, 92].

Raman is increasingly being used [93, 94]. Differences in spectra arises because of differing intermolecular interactions or molecular conformations. LOQ was found

Table 9 Examples of limits of detection (LOD) obtained by X-ray diffraction in drug substance by using one peak, Instrument Scintag XDS2000 or X_1^{TM}

Substance	LOD (peak/noise ratio >3:1)
Substance 1 [33]	A in B: 1% B in A: 2%
Substance 2 [33]	A in B: 10%
Substance 3	Beta in gamma: 2%
Trihydrate in Substance 2 [16]	1%
MKS492 [31]	1% A in B, 1% C in B, 2% D in B 1% A in C, 2% B in C, 2% D in C
Crystalline in amorphous project 1	1% crystalline
Crystalline in amorphous project 2	1% form A, 2% form B
Hydrate in anhydrous	1%

1% in case of mannitol [93]. NMR is an established technique [95]. It has been used for Neotame and formeterol [96, 97].

For the quantitative determination in formulations, the preparation (cutting, grinding) may induce transformations. Some special techniques have been proposed for X-ray diffraction [98–100]. Raman and NIR have been used in formulations [100–102]. A NIR quantitative method has been completely validated for the detection of miokamycin crystalline in amorphous myokamycin formulation down to 5% [102].

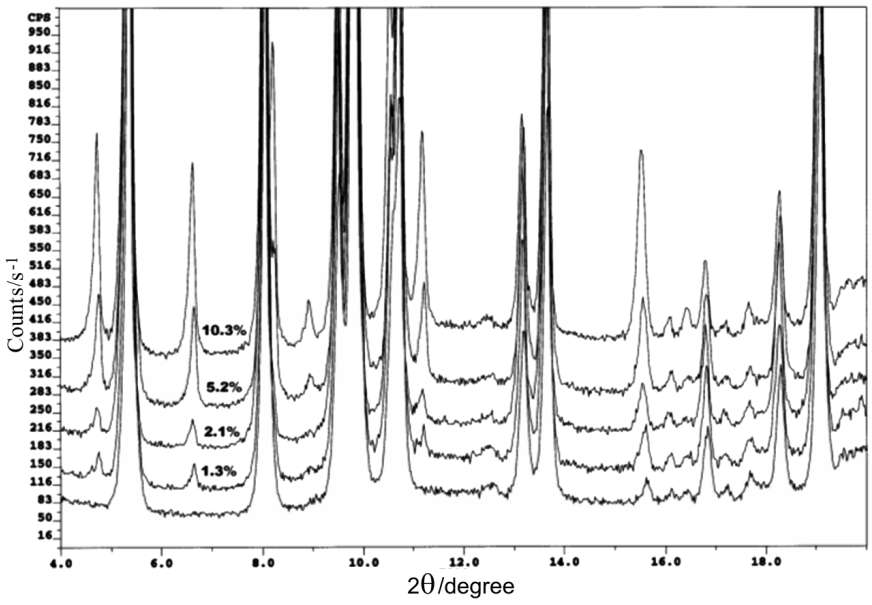

Fig. 22 Quantitative determination of a monohydrate in a tablet

Table 10 Comparison of results obtained by DSC and X-ray diffraction for the amorphous content of a new drug

Samples spiked / %	Found X-ray / %	Found DSC / %
13.6	18	21
19.7	18	21
29.2	21	26
49.6	46	47
58.5	54	50
79.4	80	74

Table 11 Comparison of results obtained by melting peak DSC, X-ray diffraction and solution calorimetry

Sample	Crystallinity X-ray diffraction / %	Crystallinity DSC / %	Crystallinity solution calorimetry / %
1	90	(90)	(90)
2	66	78	56
3	67	79	52
4	69	79	57
5	68	79	58
6	68	75	53

Infrared microimaging is now introduced for quantitative analysis of formulations [103].

New very sensitive X-ray detectors are now available, but the major issue is the homogeneity of the samples for tablets with low strengths. However, ICH Q6a is aware of the limitations for quantifying polymorphs in formulations and therefore recommends to rely on alternative methods as dissolution rate.

How to monitor the process if methods are not very sensitive? The following example (Fig. 23) illustrates a difficult case. In the crystallization/drying process a hydrated form A is obtained. The identification method is X-ray diffraction. A slight change is observed as attributed to another form B. Figure 23a shows the best X-ray diffraction peak. X-ray is inadequate for the determination of B in A. DSC shows that an endothermic peak is present only in form A and is absent in form B (Fig. 23b). The DSC can be chosen as monitoring of the process by analyzing this endotherm. Raman (Fig. 23c) shows also a difference between the two polymorphic hydrated forms. Unfortunately it is easy to determine in very small amounts of B in A but not vice-versa. The quantitative measurement of A should permit to analyse mixtures by assessment of A.

Process analytical technology is being used in-line or on-line NIR [104–107] and Raman [108–112] in manufacture of formulations or for monitoring of crystallization.

The amorphous state is desirable for poor soluble substances. However it is not desirable in processes where the crystalline state is targeted [113–115]. The determination of amorphous content in low range is a difficult task. X-ray diffraction can generally not detect lower amounts than 10%. DSC can be useful in certain cases (Table 10). Isothermal microcalorimetry is a growing successful technique for the determination of very low levels of the amorphous phases [35, 36].

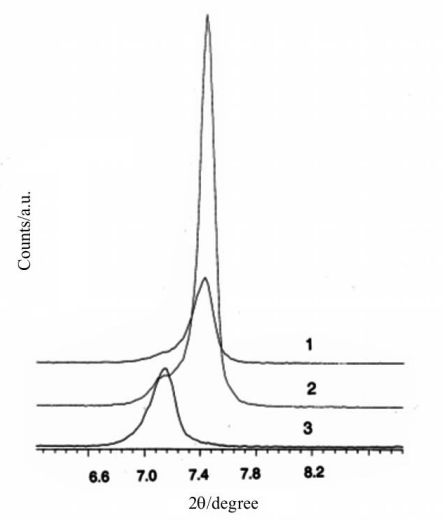

Fig. 23a How to monitor the development of a crystallization process when easy method or when pure standard are missing? The detection of B in the selected form A is desired. X-ray diffraction is inadequate to quantify B in A. 1=A, 2=sample, 3=B

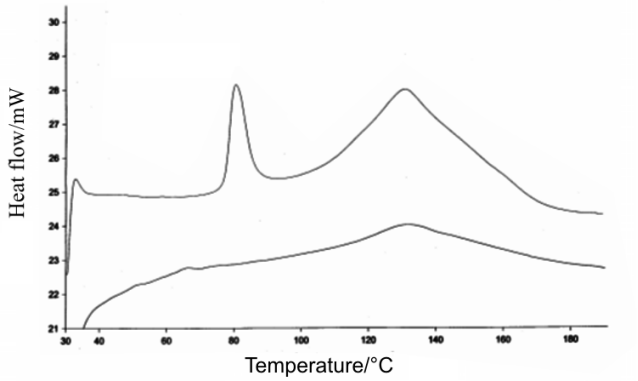

Fig. 23b How to monitor the development of a crystallization process when easy method or when pure standard are missing? The detection of B in the selected form A is desired. DSC is very discriminative, but the endothermic peak at approx. 80°C quantify A, but not B. Top: Form A, Bottom: Form B

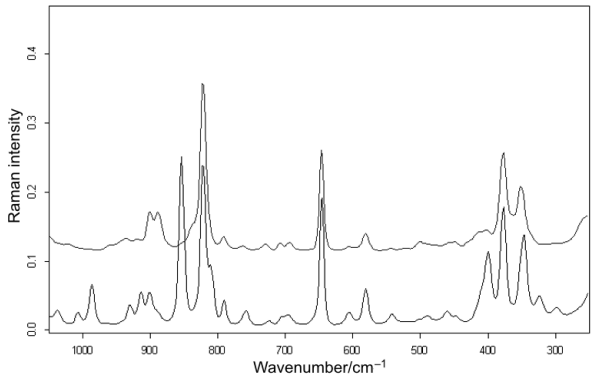

Fig. 23c How to monitor the development of a crystallization process when easy method or when pure standard are missing? The detection of B in the selected form A is desired. Raman is discriminative, but the typical peaks of B are small, while the peak of A are high: Quantitation of A in mixtures is possible. Top: Form B, Bottom: Form A

Figure 24 shows the plot of the T_g value of a drug substance with different solvents according to the Gordon-Taylor equation [116]. The measured T_g values obtained by adding water show a relatively good correlation. The decreases of the T_g is the principle of the microcalorimetric method of the determination of the amorphous part. The substance is subjected to vapors of water or organic solvent in an isothermal microcalorimeter. The peak of crystallization is measured and quantifies the amorphous content. Depending of substances, LOD of amorphous content down to 0.5–1% have been obtained [117–123].

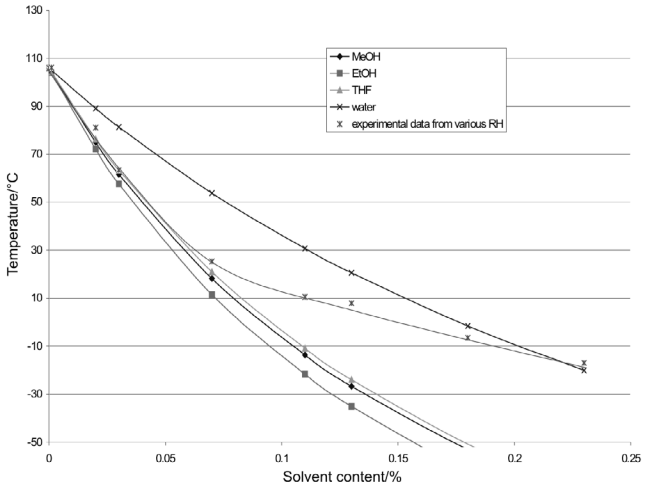

Fig. 24 Behaviour of the glass transition of a drug substance under various solvents

Tables 12 and 13 give the results of intermediate precision using dimethylformamide as solvent vapour for a compound which is amorphized by micronization (Fig. 25) [123]. A very good precision and a good accuracy are observed.

Table 12 Precision of the determination of amorphous content of a drug substance by microcalorimetry with dimethylformamide vapor

Analyst 1	
Individual values / %	16.2; 16.2;16.0; 17.2; 16.1; 16.0
Relative standard deviation:	2.8%
Mean value:	16.2% (*n*=8)
Analyst 2	
Individual values / %	15.9; 15.7

Table 13 Comparison of determination of amorphous content of a sample by using two different solvent vapors

Solvent	Ethanol/water	Dimethylformamide
Amorphous content	12.9%	12.7%

The ICH acceptance tree requires a polymorphic screening and this package is required by health authorities even for liquid formulations. During IND and for NDA methods have to be developed and results given in order to demonstrate the robustness of a process. However, if it is demonstrated that there is no relevance for the drug product, health authorities agree not to include monitoring for new entities.

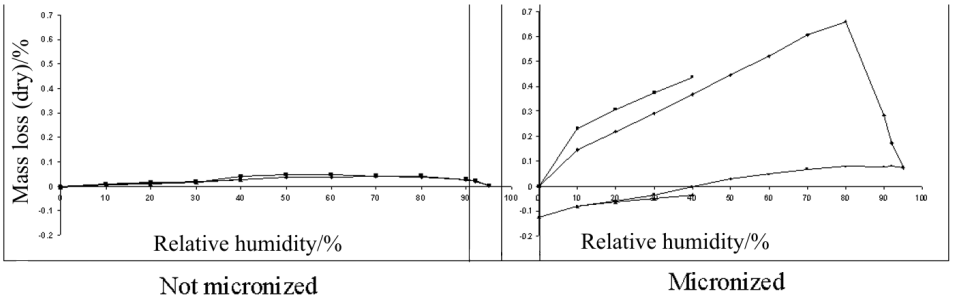

Fig. 25 Water sorption-desorption isotherms of a sample before milling and after milling. Detection of amorphization by the slight hygroscopicity observed for the sample micronized uo to approx. 80% RH. Abrupt decreases due to crystallization with water expulsion

Conclusions

For proper development and as required by ICH, polymorphism studies are part of an early drug development. High throughput technology with small sample size will speed up the selection of the solid form for new candidates.

The solid properties of new chemical entities have to be studied and characterized in the context of thermodynamic and kinetic viewpoints.

Basic thermodynamic relationships may be understood by considering the phase diagrams and the stability domains.

- Melting enthalpy rule by DSC
- Solvent mediated transformation studies
- Dissolution and solubility rule

Combined techniques allow in situ analysis in different atmospheres and temperature with a high level of information in order to obtain rapidly the proper information for the selection of the solid form of the drug substance and a proper design of the formulation.

X-ray diffraction and modeling tools have shown their efficient potential for understanding and monitoring different steps of processes from drug substance to drug product and for stability studies.

The process analytical technology for monitoring polymorphism in crystallization processes is in development.

Understanding of stabilisation mechanisms and prediction tools take an increasing part in the design of new products.

Different techniques have to be applied project specifically for quantitative methods and even low limit of detection may be necessary for critical projects. Experience show that it is not necessary to use routine methods for release when the ICH Q6 requiremens are fulfilled.

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