Journal of Thermal Analysis and Calorimetry, Vol. 64 (2001) 37–60

INVESTIGATIONS OF POLYMORPHISM AND PSEUDO-POLYMORPHISM IN PHARMACEUTICALS BY COMBINED THERMOANALYTICAL TECHNIQUES

D. Giron

Chemical and Analytical Development, Novartis Pharma AG, Basel, Switzerland

Abstract

This article summarizes the main features in the investigation of polymorphic behaviour of pharmaceuticals and emphasizes the role of combined thermoanalytical techniques for proper interpretation of complex problems. The examples illustrate the quality of information gained in relation to thermodynamic and kinetic factors.

Keywords: pharmaceuticals, polymorphism, pseudo-polymorphism

Introduction

The physicochemical properties of active pharmaceutical ingredients are key factors to the development of appropriate dosage forms. Most organic substances exist in solid state as polymorphs, pseudo-polymorphs (solvates) or amorphous forms. Since all physicochemical properties in solid state are affected mainly in terms of solubility, dissolution, bioavailability, processability and stability, it is mandatory [1] to investigate the polymorphic behaviour of active ingredients. This investigation includes the manufacture of the possible solid phases, their characterization and the knowledge of thermodynamic and kinetic factors implied in the process parameters and storage conditions of the active ingredient and of the dosage form.

Thermal analysis and microcalorimetry are the best techniques for the determination of the thermodynamic relationships between different phases: enantiotrope or monotrope transitions between true polymorphs, transitions between different solvates or hydrates and polymorphs, glass transition point of amorphous form.

Pharmaceutical compounds have often a great number of solid phases, even in metastable state and interpretations are difficult because of kinetic factors. Therefore several techniques are currently used for the study of polymorphism and pseudo-polymorphism.

Thermoanalytical techniques combining differential scanning calorimetry, microcalorimetry and thermogravimetry with microscopy, spectroscopy, X-ray diffraction or mass spectrometry are state of the art techniques. They offer quick and proper

1418–2874/2001/ $ 5.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

interpretations, they allow the manufacture of pure forms in situ, they offer the possibility of analytical quantification or crystal modeling studies.

Experimental

For the curves given in this overview, the instruments used are:

Calibrated sub-ambient Perkin Elmer DSC-7 or Perkin Elmer Pyris with robot system, calibrated TGA-7 Perkin Elmer with IR interface, calibrated TGA-850 Mettler with autosampler and with MS, calibrated SCINTAG XDS 2000 with autosampler, with heating cell and humid chamber for the X-ray diffraction experiments and a calibrated TAM (thermometric) for the microcalorimetric studies. The scanning electron microscope (SEM) is a Jeol JSM 6300 instrument, the microscope SPECTRATECH is used with the FT-IR PE-1725X and the microscope is a Zeiss Axioplan. The heating cell for the microscopes is a Mettler FP82HT. The DVS (dynamic vapour sorption) surface measurement systems is used for water sorption-desorption measurements.

Definitions

Polymorphism is the ability of a substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice [2–5]. In the solid state, the atoms, molecules or ions may be arranged in one of the fundamental crystal systems: triclinic, monoclinic, orthorhombic, tetragonal, trigonal, hexagonal or cubic. Polymorphs show the same properties in the liquid or gaseous state but they behave differently in the solid state. The most widely known example of polymorphism is the element carbon, which can exist in the form of graphite (hexagonal), diamond (cubic) or as fullerenes (C_{60} and C_{70}).

Amorphous solids are not crystalline because the arrangement of the molecules is disordered. The name ‘glassy state’ is given to amorphous products which change from glassy state to rubber state by undergoing a glass transition [6].

Solvates contain molecules of the solvent of crystallization in a definite crystal lattice [7]. Solvates, in which the solvent of crystallization is water, are termed hydrates. Because water is a constituent of the atmosphere, hydrates of drugs may be formed rather easily. The expression pseudo-polymorphism applies to hydrates and solvates.

A recent overview upon thermal analysis and calorimetric methods in the characterisation of polymorphs and solvates references more than 300 active ingredients [8].

Relevance for pharmaceuticals

All physicochemical characteristics of the solid state are involved in the polymorphism and pseudo-polymorphism. The main properties affected are melting and sublimation temperatures, heat capacity, conductivity, volume, density, viscosity, crystal

hardness, crystal shape, colour, refractive index, processability, solubilities, dissolution rate, stability, hygroscopicity and solid state reactions.

A red crystal instead of the desired yellow one is immediately detected [5], or visual differences of crystals like graphite or diamond. The scanning electron microscopy (SEM) is extremely informative for the observation of pharmaceutical powder as illustrated for an amorphous sample (Fig. 1a) and for a crystalline sample (Fig. 1b) of a drug substance.

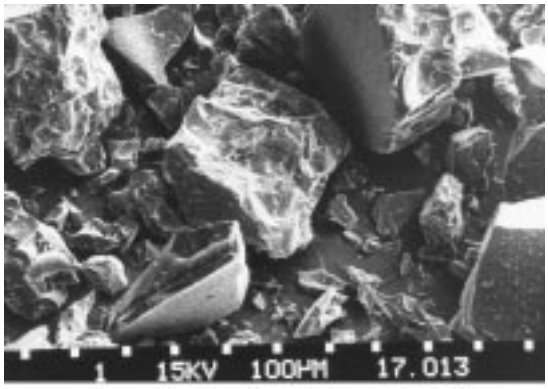

Fig. 1a SEM pictures of polymorphs of a drug substance, amorphous form

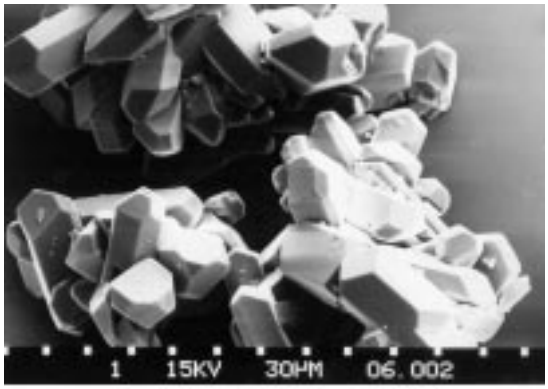

Fig. 1b SEM pictures of polymorphs of a drug substance, one crystalline form

The effect of polymorphism on bioavailability or toxicity [2, 3, 9,10] is the most important consequence for drug substances if the bioavailability is mediated via dissolution. For the most famous case of chloroamphenicol palmitate [3, 45], the active polymorph is not the thermodynamical stable one. The polymorphism of the excipients may also play an important role in bioavailability [11].

Figure 2 is an example of polymorphism associated with a tremendous effect of dissolution for the active substance and for the drug product giving rise to a change of bioavailability.

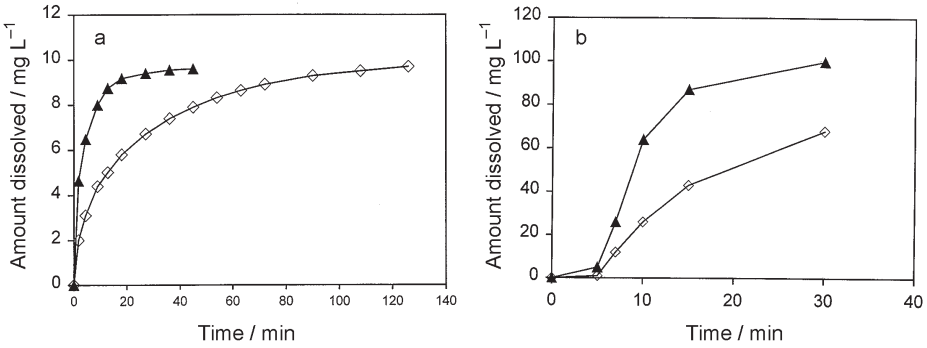

Fig. 2 Dissolution rate curves of two crystalline modifications (A and B) of a drug candidate and the corresponding curves of the drug products consisting of capsules containing A and B. a – dgug substance form A: –▲– form B: –◇–; b – capsules form A: –▲– form B: –◇–;

Chemical reactivity in the solid state is correlated with the nature of the crystalline modifications. Walkling [12] found that the two crystalline modifications of fenretinide behave quite differently. After 4 weeks at 25°C, the stable form showed no detectable degradation, whereas the unstable form showed 8% degradation. In a recent example, the hydrolysis of an investigational compound led to a toxic degradation product for which one solid form was much less stable than the other [13].

The amorphous state is very reactive. According to Zografi [6, 14], the chemical reactivity in the liquid state should enable us to predict that of the amorphous state.

Table 1 illustrates the differences of reactivity of two crystalline polymorphs (example 1) and the difference of reactivity of a crystalline form and the amorphous form of a peptide drug candidate (example 2).

Table 1 Examples of stability behaviours of crystalline and amorphous forms

Example 1. Degradation after 1 month stress at 80°C under oxygen or moisture	
Crystalline form A	no degradation
Crystalline form B	0.5–1.5% degradation
Amorphous form	2–3.5% degradation
Example 2. Degradation after 1 week at 70°C	
Crystalline form	10% degradation
Amorphous form	80% degradation
Example 2. Degradation after light exposure, 300 kluxh	
Crystalline form	2% degradation
Amorphous form	38% degradation

Since processing and storage imply changes of temperature, pressure and humidity, polymorphic transitions are undesirable phenomena. For example polymor-

phic transitions may be induced by mechanical processing [15, 16]. Furthermore for kinetic reasons thermodynamically unstable forms named metastable forms may exist outside phase diagrams equilibrium curves. Metastable forms may be manufactured on purpose but aging problems are observed when metastable forms undergo transitions into stable forms according to thermodynamic rules.

A solvate may first be crystallized and then transformed upon drying or mechanical process into a metastable anhydrous or desolvated form. In this respect water activity in organic solvents is the critical parameter of formation of hydrate forms.

Water being a component of the atmosphere which considerably vary from country to country and day to day is the most critical parameter when substances may transform into hydrates under normal storage conditions. Water will be absorbed and desorbed with temperature and moisture changes. The crystallization of theophylline monohydrate in tablets is a classical example [17]. Additionally to physical changes, free water may react chemically. Kankari and Grant recently reviewed pharmaceutical hydrates [18].

Typically obtained during lyophilisation, spray-drying, granulation, grinding or milling, the amorphous form is responsible for the higher reactivity of some batches. It gives rise to major problems with activity and stability. Amorphous forms generally tend to crystallize in the presence of moisture (e.g. indomethacine, lactose [14]). Batch to batch reproducibility also affects storage behaviour, milling ability and crystalline transitions during processing of the dosage forms.

Ageing problems after storage are observed for solutions, microemulsions, lyophilisates, creams, suppositories, MR forms, solid dispersions, powder for inhalation, capsules, tablets [8, 9, 11].

The following example corresponding to Fig. 2, deals with the transformation of one batch of the metastable form A into the stable form B while the other batch remains unchanged. Figure 3 is the plot of the content of the stable form B in the two batches of metastable form A *vs.* time after storage at 30°C. No transformation takes

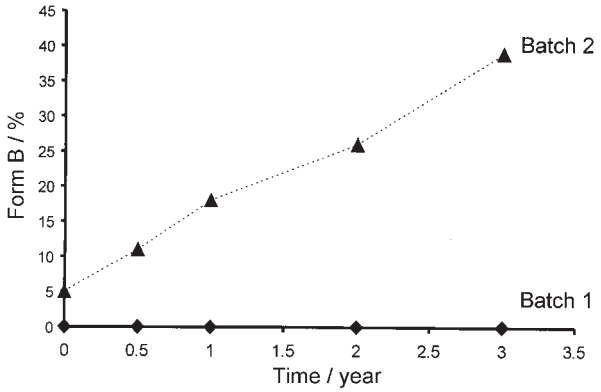

Fig. 3 Content of the stable form B in two batches of metastable form A after storage at 30°C determined by X-ray diffraction. A transformation is observed only in the second batch containing traces of form B

place in the one batch which does not content seeds of form B. In contrary only 5% seeds of form B were found responsible to the quick transformation of form A into form B for the second batch. This example [19] emphasizes the need for very sensitive analytical methods (e.g. X-ray diffraction in this case) in the investigation of polymorphs.

Thermodynamic and kinetic aspects

The relationships between different phases of a substance are governed by Gibbs' phase rule:

$$P+F=C+2 \quad (1)$$

where C is the number of components, P is the number of phases that exist in equilibrium, and F is the number of degrees of freedom, i. e., the variance, of the system.

In the case of a single substance exhibiting polymorphism, C equals unity. If one phase, i.e., one polymorph, is present, P=1, therefore F=2. Equation 1 reveals that the variance, F=2, meaning that both temperature and pressure may be varied without changing the number of phases. If two phases, i. e. two polymorphs, are in equilibrium, P=2, in which case the variance F=1, meaning that, at a chosen pressure, usually atmospheric pressure, the temperature of the system is fixed at the so-called transition temperature, T_t. The conclusion from the phase rule is that only one phase can exist at any given temperature and pressure, except at the transition temperature at a defined pressure, usually atmospheric, in which case two phases, e. g., polymorphs, exist in equilibrium.

The process of transformation of one polymorph into another is a phase transition, which, according to the phase rule, may occur at a given pressure by changing the temperature. If the phase transition is reversible, the two polymorphs are *enantiotropes* and the energy of the transition on heating is endothermic. If the phase transition is irreversible, the two polymorphs are *monotropes*, in which case only one form is stable whatever the temperature and the transformation of the unstable form to the stable one is exothermic.

For kinetic reasons, an unstable form may exist for a time outside the region assigned by the phase diagram and the phase rule, and is then termed a *metastable* form.

The ability of a system to perform work and to undergo a spontaneous change at constant pressure is measured by the Gibbs free energy, G, which is given by

$$G=H-TS \quad (2)$$

In general, the thermodynamic relationship between two polymorphic phases is represented by plotting the Gibbs free energy as a function of temperature for each form (Fig. 4). At a given temperature, if the two curves intersect below the melting point of each polymorph, a reversible transition occurs at the temperature T_t of the intersection. At temperatures below T_t, polymorph A has the lower free energy and is therefore the thermodynamically stable form, while at temperatures above T_t poly-

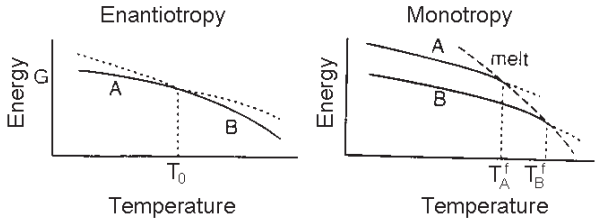

Fig. 4 Gibbs free energy diagrams in case of enantiotropy and monotropy

morph B is stable. In the case of monotropy, the higher melting form is always the thermodynamically stable form.

Burger [20–22] proposed to plot *energy diagrams* (Fig. 5) showing the free energy and the enthalpy, as functions of temperature. As shown in Figs 5a and 5b, a notable difference between enantiotropy and monotropy is the melting enthalpy of the higher melting form. In the case of enantiotropy, the *higher* melting form has the

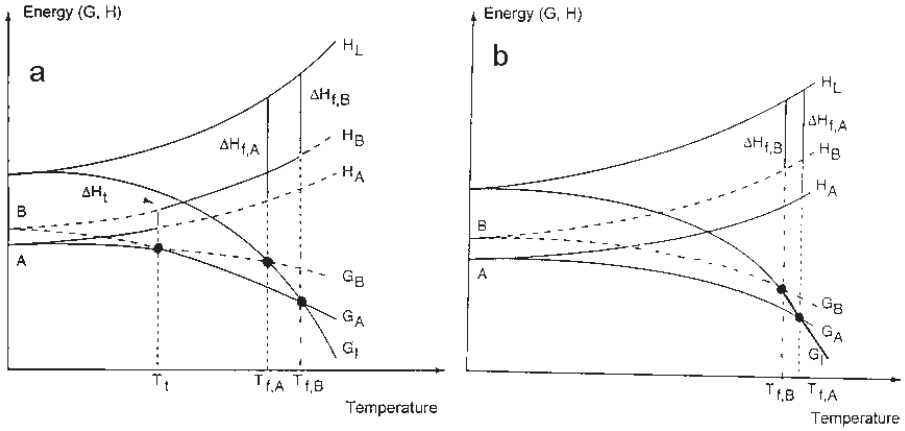

Fig. 5 Energy diagrams showing plots of enthalpy, *H*, and Gibbs free energy, *G*, *vs*. temperature, *T*, for the solid and liquid phases of a single compound, showing a – enantiotropy, and b – monotropy

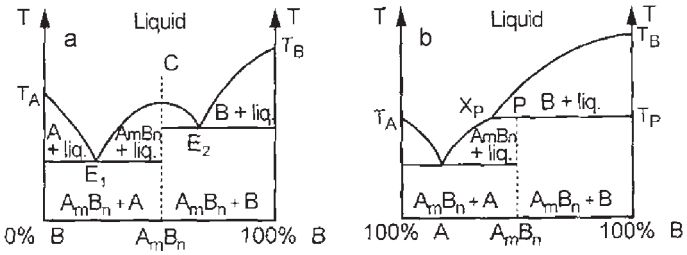

Fig. 6 Phase diagrams of binary mixtures temperature *vs.* composition (e.g. mole fraction) in case of solvate formation. a – formation of a compound with a congruent melting point at C, b – formation of a compound with an incongruent melting point at P

lower melting enthalpy. In the case of monotropy, the *higher* melting form has the *higher* melting enthalpy.

Solvates show interesting contrasts, because there are two components, the host and the solvent. The phases to be considered are the solvate, the unsolvated host, and the solvent. In the more complex cases of several solvates, the different phases with different compositions have their respective domains of stability. Figure 6 shows two typical phase diagrams of solvates. Congruent or incongruent melting of the solvate may be observed, leading to a mixture of the two separate components. The phase di-

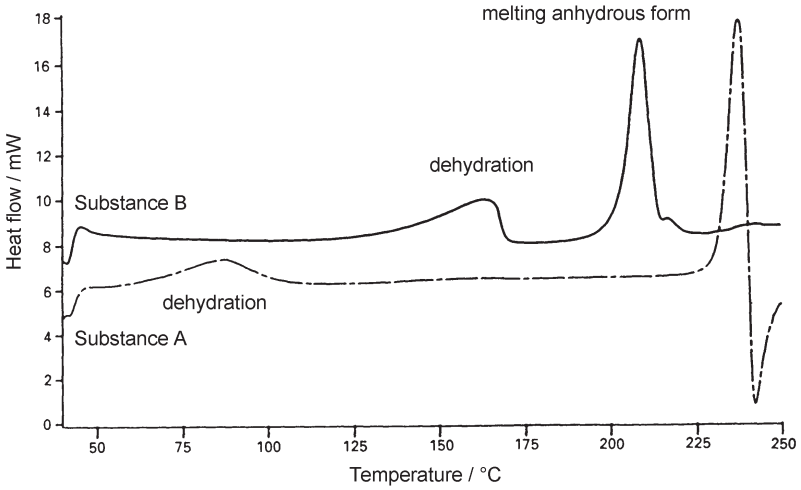

Fig. 7 Different DSC dehydration temperatures resulting from phase diagrams

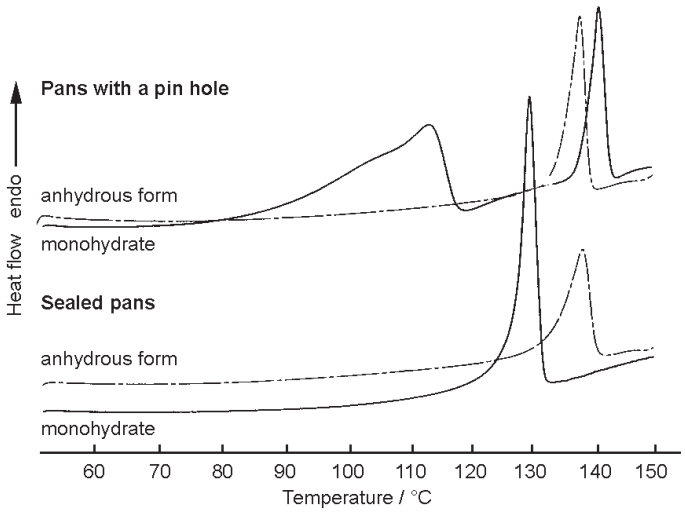

Fig. 8 Influence of the sample types on the DSC curves of a hydrate

agram of solvates is responsible of the different behaviour of desolvation observed in DSC as examplified in Fig. 7. Depending on the pan type used for the DSC, the solvent may escape or not before the melting: with a sealed pan, the melting peak of a hydrate can be measured (Fig. 8). Polymorphism can occur between solvated forms [8]. Furthermore several hydrates may exist with different domains of thermodynamic stability. When considering the relation solubility/temperature, lower is the temperature, higher is the number of molecules of solvent or water bounded and lower the solubility. For example, ouabaine was obtained as an anhydrous form, dihydrate, trihydrate, tetrahydrate, octahydrate and even as a nonahydrate depending of the conditions of manufacture [4].

Because process development aims at a robust process, it is mandatory to evaluate the tendency of solvate formation during the crystallization procedure. If solvent mixtures, e.g. aqueous alcohols, are used, the formation of anhydrous form(s), solvate(s), and hydrate(s) have to be considered and the stability zones, with respect to temperature and solvent composition, have to be determined.

As mentioned above, the formation of several hydrates of a given compound frequently occurs. If the temperature is varied over a wide range, a series of equilibria will be observed. Soustelle [23] discussed the implication of water vapour pressure on the system comprising the hydrates of copper(II)sulphate as illustrated in Fig. 9a.

– At pressures below P1, only one equilibrium is possible, that between the pentahydrate and the anhydrate. The corresponding curve representing the water con-

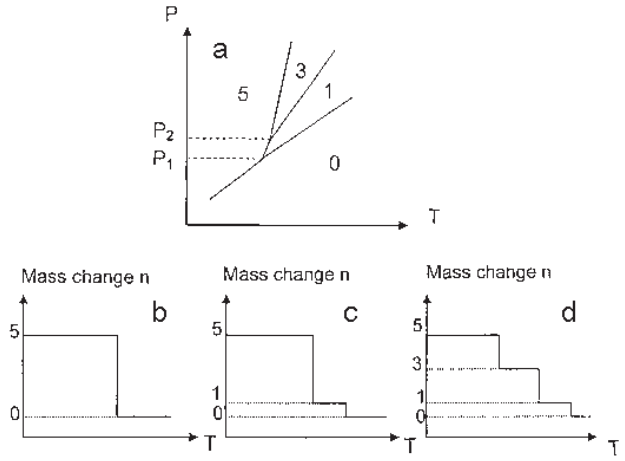

Fig. 9 Influence of pressure and temperature on the composition of the stable phases formed between copper(II) sulfate and water (after Soustelle, [23]). *n* indicates the number of moles of water associated with copper(II) sulfate, i.e. $CuSO_4 \cdot nH_2O$. a – Phase diagrams of pressure *vs*. temperature showing the equilibrium curves of the anhydrate (*n*=0), monohydrate (*n*=1), trihydrate (*n*=3) and pentahydrate (*n*=5). b – Thermogravimetric curve obtained at a constant pressure, P, below P1. c – Thermogravimetric curve obtained at a constant pressure, P, between P1 and P2. d – Thermogravimetric curve obtained at a constant pressure, P, above P2

tent as a function of temperature, determined by thermogravimetry, is shown in Fig. 9b. A mass loss of 36.05% corresponding to 5 mol water occurs.

– At pressures between P1 and P2, the corresponding thermogravimetric curve, shown in Fig. 9c, involves the phase transformation from the pentahydrate to the monohydrate, followed by the transformation of the monohydrate to the anhydrate with the corresponding mass loss of 28.84 and of 7.21% referring to the pentahydrate. At the triple point, P1, both equilibria occur simultaneously.

– At pressures above P2, the thermogravimetric curve corresponds to the three successive monovariant transformations, pentahydrate $\rightarrow$ trihydrate $\rightarrow$ monohydrate $\rightarrow$ anhydrate, (Fig. 9d) with the mass loss of 14.42, 14.42 and 7.21%.

If phase transformations were based solely on thermodynamic rules, stable crystal forms should be obtained quite easily. However kinetic factors cause metastable states to be encountered. A considerable activation energy barrier may have to be overcome in moving from a metastable state to a stable state. The activation energy, E, is related to the rate constant, k, of the transformation by the following well-known Arrhenius equation:

$$k=Ae^{-E/RT} \tag{3}$$

where A is the frequency factor, which is related to the entropy of activation, R is the universal gas constant and T is the absolute (Kelvin) temperature. The activation energy barrier may be reduced by catalysts, impurities, and/or crystal defects. When comparing the stability of solids during storage, many factors play a role, such as temperature, particle size, the presence of seed nuclei of the product, activation energy for the change, and diffusion of the molecules.

Transformations may be accelerated by the presence of a solvent, such as water. *Solvent mediated transformations* occur by a continuous dissolution-crystallization process. This type of transformation may occur during crystallization or granulation. Hydrates may be formed by this process in mixtures involving moisture. Solvent me-

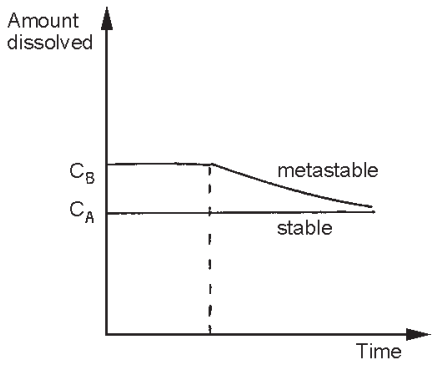

Fig. 10 Solubility curves of stable and metastable forms. The solubility of the metastable form decreases resulting from the transformation into the stable form. The transformation is accelerated in suspensions via solvent mediated transitions

diated transformations may occur when measuring solubilities, such that, after some time, recrystallization of a more stable state may be complete (Fig. 10).

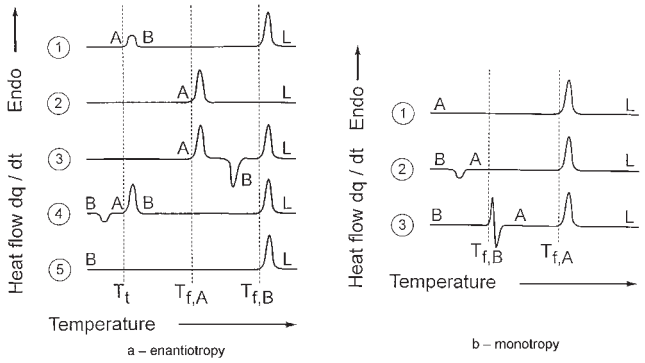

Fig. 11 DSC curves which may be observed in case of enantiotropy (Fig. 9a) or monotropy (Fig. 9b)

For all analysis where a temperature change is involved, *kinetic factors* have to be considered for proper interpretation of the results as demonstrated in Figs 11a and 11b. The DSC scans will differ if the sample being analysed is stable or metastable at ambient temperature [24].

Case of enantiotropy (Fig. 11a)

Scan 1. The sample studied is the stable form A, which gives the endothermic solid phase transition A→B followed by the melting endotherm of form B.

Scan 2. The sample studied is the stable form A, but for kinetic reasons, e. g. a too fast heating rate, the solid transformation A→B does not occur. Instead, form A melts.

Scan 3. The sample studied is the stable form A which melts. Form B grows from the melt with an exothermic peak and form B melts at a higher temperature.

Scan 4. The sample studied is the metastable form B, which becomes stable at a higher temperature above the transition temperature. An exothermic peak corresponds to the solid transformation B→A followed by successive transformation A→B and melting of B.

Scan 5. The sample studied is the metastable form B. The DSC scan shows its melting endotherm.

Case of monotropy (Fig. 11b)

Scan 1. The sample studied is the stable form A and its melting endotherm is observed.

Scan 2. The sample studied is the metastable form B which transforms exothermically in the solid state into the stable form A. Form A melts at a higher temperature.

Scan 3. The sample studied is the metastable form B, which does not transform into A but melts endothermically. From the melt, the stable crystalline form A is obtained with an exothermic peak and melts at a higher temperature.

The same interpretations apply to all methods that involve heating (e.g. hot stage optical microscopy, hot stage infrared or Raman microscopy, temperature resolved or variable temperature X-ray diffractometry).

For characterization of polymorphs, the influence of heating rates, cooling rates of pure forms and of mixtures of polymorphs should be studied. By using fast heating rates, it is possible to obtain the melting peak of metastable forms and to calculate the corresponding melting energy. The melting energy of the high melting forms is easily calculated by applying slow heating rates or by tempering the substance in situ. The application of the Burger's rule [20–22] allows to know if the relation between polymorphs is enantiotropic or monotropic. The example of butylhydroxyanisole illustrates such a study [11, 45]. The commercial sample shows only one peak with a melting energy of 103 J g^{-1} with the heating rate of 10 K min^{-1}. The higher melting form is obtained by the heating rate of 2.5 K min^{-1}. and the melting enthalpy is found 87 J g^{-1}. Therefore, it is concluded that both forms are enantiotropically related. The lower melting form is the stable form at ambient temperature.

Combined techniques

A comprehensive characterization of the physical properties of materials often requires a multi-disciplinary approach since no single technique is capable of characterizing pharmaceuticals completely. DSC and TG are very sensitive, but are not specific, the solid transformations may have too low energies, impurities have a high impact on the melting points [25] and the amorphous content influences the measured melting enthalpies.

Thermomicroscopy or hot stage microscopy is a well established method [26–28] for the observation of the sample while heating or cooling, allowing to see desolvatation, melting, crystallization, eutectic formation and even transformations in suspensions in solvents. The combination of hot stage microscopy to new technol-

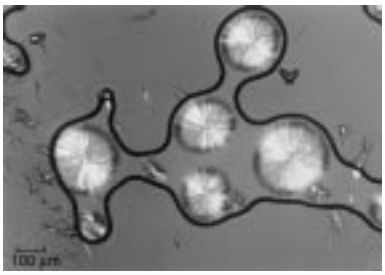

Fig. 12 Melting/crystallization observed under polarized light

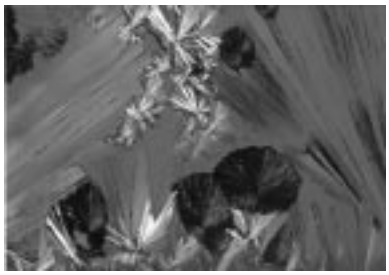

Fig. 13 Propylphenazone. The two enantiotropically related forms may be obtained simultaneously by fast cooling from the melt as observed by thermomicroscopy

ogy such as high resolution color camera, image manipulation software makes the technique very attractive for inducing metastable states, for observation of crystal habit and for better interpretation of other methods. Figure 12 shows an example of melting/crystallization observed under the thermomicroscope with polarised light. Figure 13 shows the formation of the metastable form of propylphenazone by cooling the melt. In this case of enantiotropy, both forms are observed by fast cooling.

FT-IR microscopy [29], Raman-microscopy [30, 32] are excellent additional tools to thermomicroscopy.

Temperature resolved X-ray-diffraction with a heating cell is widely used [33–35]. Crystalline changes are clearly assigned, the X-ray diffraction pattern obtained in situ allow to predict quantitative methods if, for kinetic reasons, forms which are present at high temperatures occur at ambient conditions. Low temperature X-ray diffraction cell has been developed for the study of frozen aqueous solutions [36]. The introduction of XRD-DTA cell [37] and recently of the DSC-XRD instrument of Rigaku presented at the Denver X-ray Conference in 1999 [38] demonstrates the advantage of this direct combined technique. The observation of polymorphic transformation by using variable temperature synchrotron X-ray diffraction method is a promising technique with the new computerized ability for obtaining structural data [39].

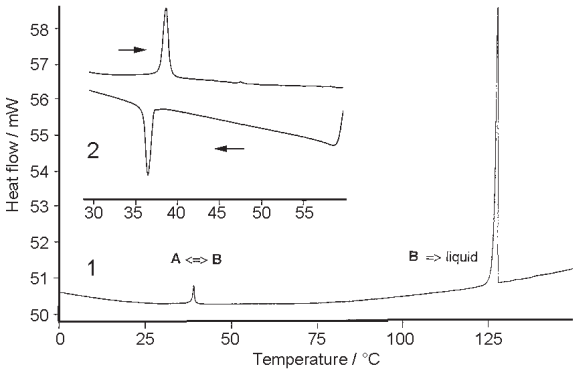

Fig. 14 DSC study of the reversible solid state transition of tolbutamide. 1 – DSC scan at 10 K min^{-1}. 2 – heating and cooling cycles between 30 and 60°C

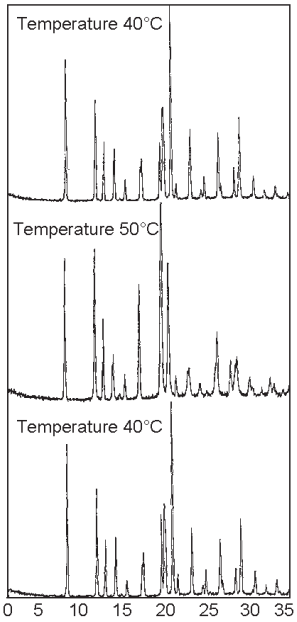

Fig. 15 Temperature resolved X-ray diffraction of tolbutamide heating and cooling between 40 and 50°C

Figure 14 deals with the DSC scans of the commercial tolbutamide. In Fig. 14.1, a first small endotherm is observed at about 40°C followed by a second sharp melting endotherm at 126°C [40]. The reversibility of the transition A⇔B is easily demonstrated by DSC (curve 14.2). The same energy is found even after repeated heating-cooling cycles. Figure 15 shows the advantage of the temperature resolved X-ray diffraction which shows that this endotherm is due to a reversible phase transition.

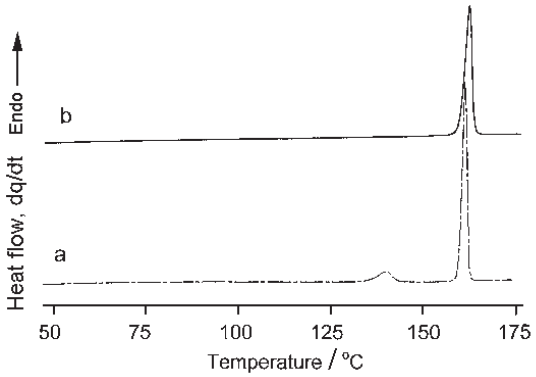

Fig. 16 DSC curves of two polymorphs, B (a) and A (b), of oxybuprocaine hydrochloride. Polymorph B undergoes an enantiotropic transition to polymorph A

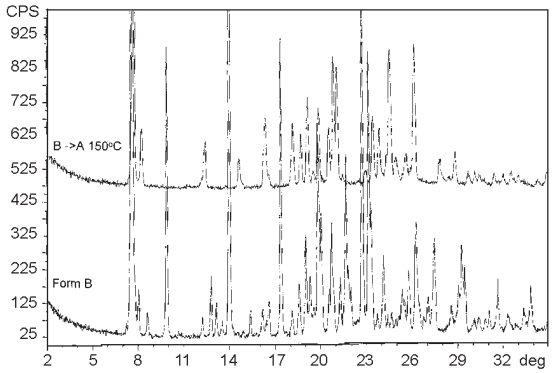

Fig. 17 Oxybuprocaine hydrochloride. X-ray diffractions of form B before and after the transition into form A

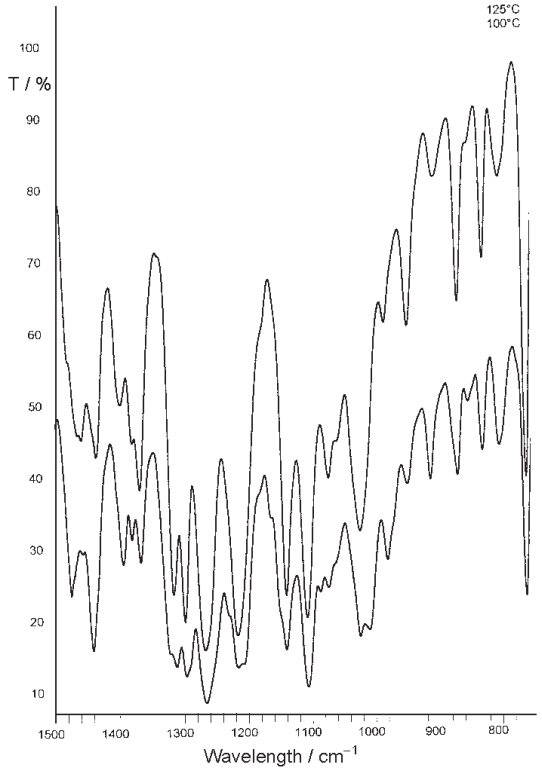

Fig. 18 Oxybuprocaine hydrochloride. FT-IR microscopy study of the transition B→A

The X-ray diffraction of the form B can be measured only at temperature above the transition point.

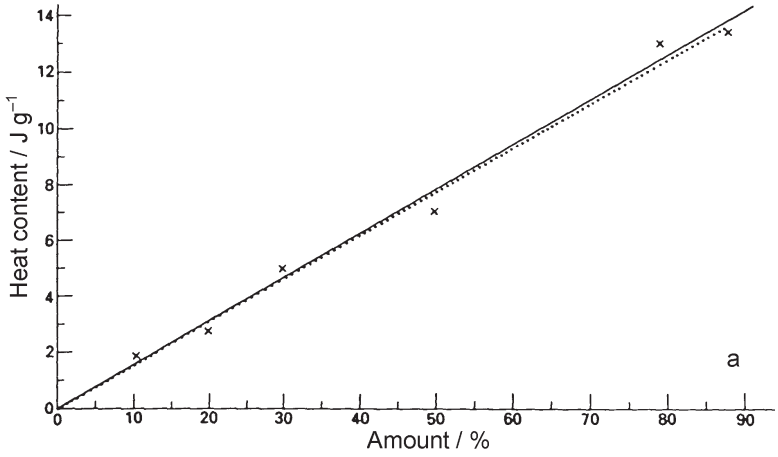

Fig. 19 Oxybuprocaine hydrochloride. Quantitation of polymorphic mixtures using the endothermic transition. Correlation coefficient, r=0.995

Figure 16 represents the DSC scans of two batches of Oxybuprocaine hydrochloride. We studied this enantiotropic behaviour some years ago [8, 41]. The DSC scan of USP reference did not show the small endotherm of about 15.6 J g^{-1} corresponding to the enantiotropic transition observed in the stable form. The high melting form (DSC onset 159°C, melting enthalpy 106 J g^{-1}) should be stable only above the transition point, however under cooling no transformation was observed. Samples of modification A or mixtures of A and B were stable. The dissolution of both forms were found very similar. Figure 17 shows the X-ray diffractions of form B at ambient temperature and after 150°C. The new X-ray pattern corresponded to that pattern of the USP reference. The IR spectra were also different and the transition B→A can be also followed by FT-IR microscopy (Fig. 18). A quantitation of form B in mixtures A+B was found possible by using the endotherm of transition (Fig. 19 [8]).

The study of *solvent mediated transitions* by using each polymorph or mixtures of polymorphs is a very efficient tool for the determination of thermodynamic rela-

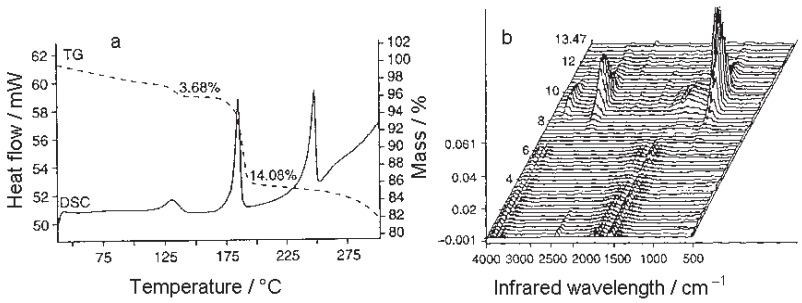

Fig. 20 Use of combined DSC and TG-IR for Aspartam

tion between polymorphs. This was successfully applied for oxybuprocaine hydrochloride as well as for other polymorphic investigations [35, 42].

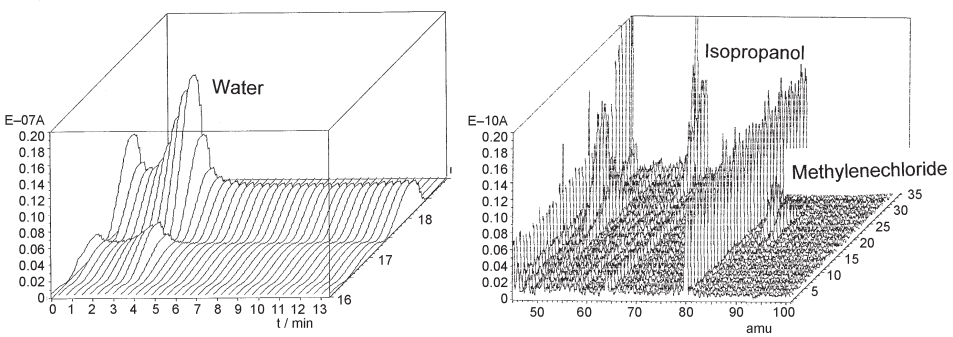

Fig. 21 Use of combined TG-MS and discrimination between water bound as hydrate and encaged solvents methylenechloride and isopropanol

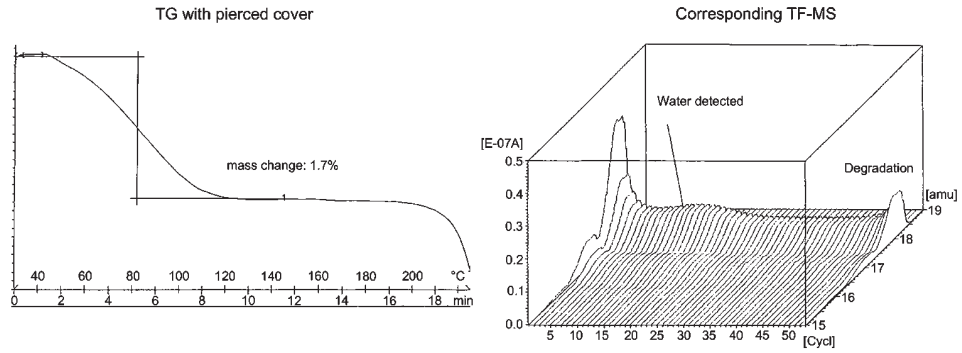

Fig. 22 TG-MS of the monohydrate of a drug substance

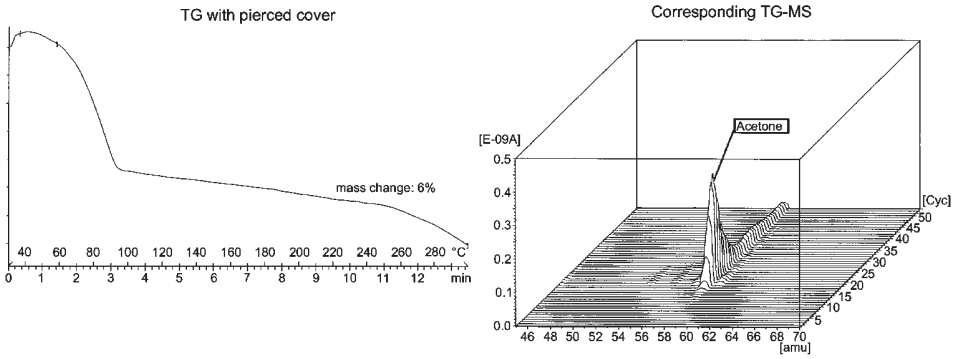

Fig. 23 TG-MS of the acetone solvate of the drug substance given in Fig. 22

The characterization of solvates and hydrates need the use of both DSC and TG. Desolvatation can be complex: melting, or solid state transformation with evaporation and possibly further endothermic or exothermic events corresponding to a cascade of phase transitions. In such complex situations, *combined techniques TG-IR or TG-MS* are extremely helpful since the identity of the volatile component is determined *in situ*.

Figure 20 deals with the TG-IR of aspartam [43]. The first component evolved is water corresponding to the dehydration of the hemi-hydrate. The second component is methanol as resulting of decomposition of aspartam. The last DSC peak is the melting of the cyclic piperazine formed.

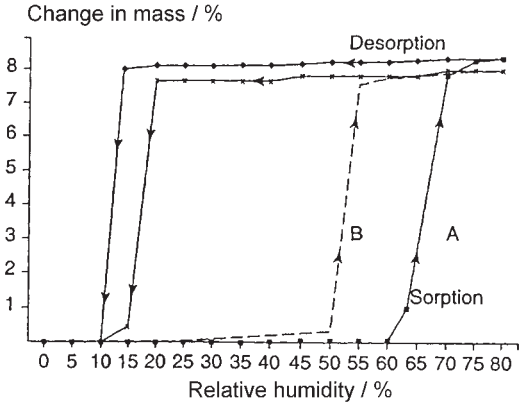

Fig. 24a Water sorption/desorption isotherms combined with moisture X-ray diffraction. Hygroscopicity and polymorphs. The two polymorphs, A and B, transform into the same hydrated form. The metastable form B takes up water at lower RH than the stable form A. The hydrate form loses water at RH values below 20%

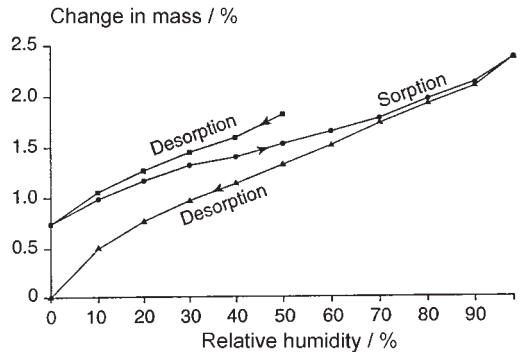

Fig. 24b Water sorption/desorption isotherms combined with moisture X-ray diffraction. The acetone solvate is transformed in the hydrate during the water sorption

Figure 21 shows the example of a compound obtained by precipitation through addition of co-solvent. A first DSC peak is correlated with the dehydration of the hydrated form. Methylenchloride and isopropanol were further evolved during melting.

Figures 22 and 23 correspond to the TG-MS of the monohydrate and the acetone solvate of a drug substance.

The study of hydration, dehydration of drug substances is an important part of the investigations to be carried out with new substances. This is achieved by measuring the *water sorption-desorption isotherms*.

Examples of such measurements are given in Figs 24a and 24b. Figure 24a shows the different behaviours of two polymorphs, enantiotropically related, of a drug substance. Both take up water and transform into the hydrated form, but at different relative humidities (R.H.). There is a considerable hysteresis in the dehydration. This experiment can be followed by X-ray diffraction. In the example of Fig. 24b, a solvate was transformed into a hydrated form.

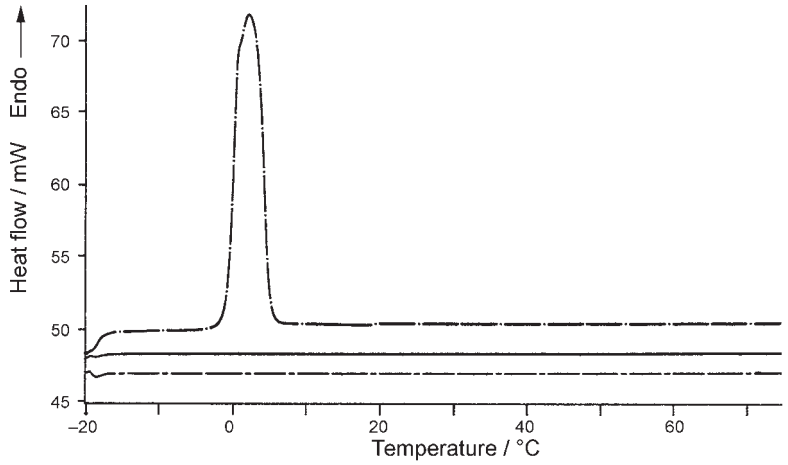

Fig. 25 Study of hydrates by sub-ambient DSC combined with TG and X-ray diffraction. From bottom to the top: DSC of the drug substance, DSC of the drug substance after storage at 92% RH. DSC of the drug substance suspended in water. Measurement of the amount of freezable water. By TG measurement of the total amount of water. Calculation of bound water: 3.5%, Monohydrate: theoretical mass 3.5%

Depending on the phase diagrams, solvates or hydrates may be partially dissociated at ambient conditions. The determination by sub-ambient DSC of the freezable water of substances after suspension in water has been suggested for the determination of the number of molecules of water bounded to the drug substance [46] as demonstrated in Fig. 25 for a monohydrate. TG analysis allows to determine the total content of water.

Suryanarayanan *et al.* [47] studied the dehydration of ampicillin trihydrate by pressure differential scanning calorimetry and variable temperature X-ray diffrac-

tion. The dehydration and the vaporization endotherms of water could be separated enabling quantitative determinations. Depending on the pressure amorphous or crystalline ampicillin were obtained.

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 slope occurs at the so-called *glass transition temperature* T_g. The glass transition is characterized by a change of heat capacity. 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. The use of amorphous forms is attractive, particularly for sparingly soluble compounds because of the enhanced solubility and dissolution rate over the crystalline state leading to increased bioavailability. However, the amorphous state is thermodynamically unstable. The glass transition temperature, T_g, is lowered by water or other additives according to the Gordon–Taylor Eq. (4), facilitating conversion to the rubbery state and hence facilitating crystallization.

$$T_g = w_1 T_{g_1} + K w_2 T_{g_2} / w_1 + K w_2 \quad (4)$$

Since it may be interesting to maintain the amorphous state, the temperature of the glass transition and its behaviour should be characterized. DSC and modulated DSC are commonly used. Zografi *et al.* studied intensively the amorphous state of drug substances and used the relaxation energy at the glass transition as well as the dependency of the heating rate for the study of the 'fragility' of the amorphous state [6, 14].

Considering that water decreases the glass transition temperature, allowing fast crystallizations, Byström [48] developed a determination of the *amorphous content*

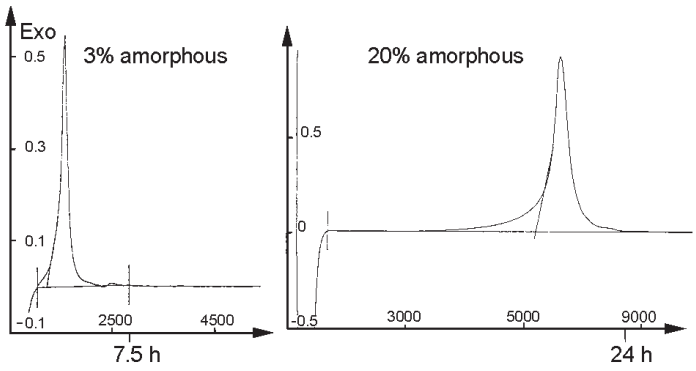

Fig. 26 Use of microcalorimetry for the determination of amorphous content. Comparison of the microcalorimetric curves and comparison of the time of analysis required for samples with 3% amorphous content and 20% amorphous content

of drugs by the measurement of the energy of crystallization of the sample stored under high humidity by *isothermal microcalorimetry*. As pre-requisite, the amorphous substance adsorbs water, the glass transition decreases and the crystallization in crystalline state occurs. Depending of the temperature different polymorphs may be obtained. Limits of detection of 1–2% could be obtained easily [42, 49, 50]. The measurement of the water adsorption/desorption energies are not always separated from the crystallization, but the whole energy measured is proportional to the amorphous content. The temperature of measurement, the humidity level and the amount to be measured are parameters to be considered in order to carry out analysis in a reasonable time range as demonstrated for Desferal in Fig. 26. Unfortunately the method requires a long time of analysis. The use of X-ray diffraction is very convenient for routine analysis of the amorphous content, but is generally limited to approx. 10%.

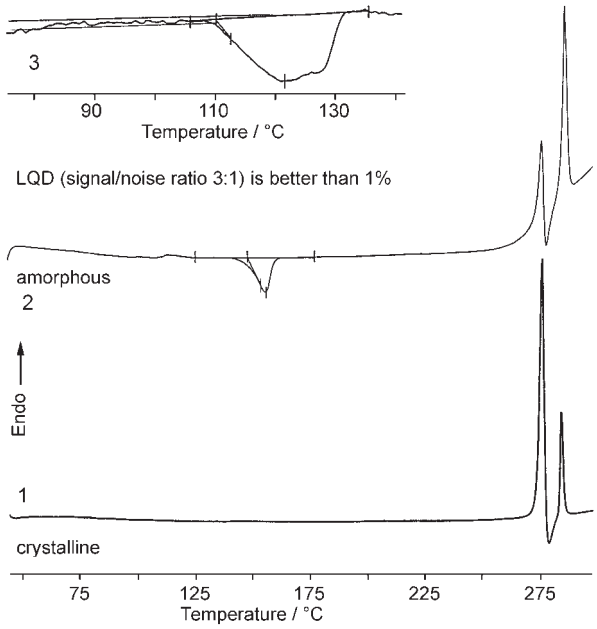

Fig. 27 Comparison of DSC and X-ray diffraction for the determination of the amorphous content. The crystallization exotherm occurs by DSC and can be used for determination down to 1%.

Figure 27 shows the DSC scan of a crystalline drug substance (27.1) with enantiotropic behaviour and the DSC scan of an amorphous sample (27.2). Very small exotherms were observed in DSC of samples micronized or lyophilized. The enthalpy of the exothermic crystallization has been suggested for quantitation purposes. The amorphous form was manufactured and quantitative methods developed by using X-ray diffraction and DSC. The linearities were checked for both methods: correlation coefficients of about 0.99 were found. The limit of detection was found 5% by X-ray diffraction and 1% by DSC. The comparison of DSC and X-ray diffraction re-

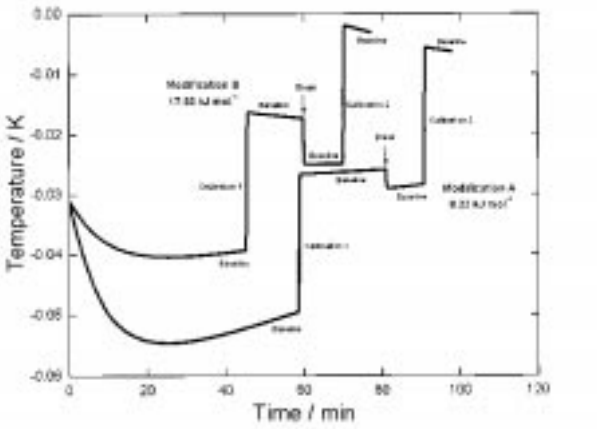

Fig. 28 Example of the use of solution calorimetry for the study of two polymorphs

sults was reliable in range >20% amorphous. This DSC measurement is quite quicker than the microcalorimetric determination and can be taken into consideration for routine analysis if no kinetic factor occurs.

The *heats of solution* of substances can be measured directly by solution calorimetry. Quantitative analysis of polymorphs, solvates and amorphous forms in mixtures has been performed using solution calorimetry [8]. The difference between the heats of solution of the two polymorphs is approximately equal to the transition enthalpy of the polymorphs at temperatures close to ambient. The results provide an alternative to DSC for the discrimination between enantiotropy and monotropy, when the substance decomposes upon melting, if the temperature of melting is known. Figure 28 shows the measurements of the energy of solution in water of the two modifications of a drug substance. The difference of 9.7 kJ mol^{-1} is very close to the difference of the melting energies measured by DSC of 9.1 kJ mol^{-1}.

Conclusions

Polymorphism and pseudo-polymorphism are frequent and highly relevant for pharmaceuticals. Since kinetic factors are responsible to the presence of metastable forms, the thermodynamic relationships in function of the temperature, pressure, humidity, solvents, process parameters and storage conditions need to be investigated in depth and several methods are necessary. Thermoanalytical methods which are related to thermodynamic data play a key role. Emerging technologies combinining thermal and calorimetric methods with microscopy, spectroscopy, X-ray diffraction and mass spectrum offer quicker ways for proper interpretation of polymorphic phenomena, but the sensitivity, the reliability and the robustness of the results should remain of high quality.

* * *

Many thanks to C. Goldbronn, M. Mutz, S. Pfeffer, P. Piechon and F. Zaman to their contribution.

References

1 International Conference on Harmonization (ICH), Guideline Specifications Q6A, 1999.
2 J. Haleblian and W. McCrone, J. Pharm. Sci., 58 (1969) 911.
3 J. Haleblian, J. Pharm. Sci., 64 (1975) 1269.
4 S. R. Byrn, Solid State Chemistry of Drugs, Academic Press, New York 1982.
5 G. R. Desiraju, Organic Solid State Chemistry, Elsevier, Amsterdam 1987.
6 B. C. Hancock and G. Zografi, J. Pharm. Sci., 86 (1997) 1.
7 R. K. Morris, Pharmaceutical Solids, H. G. Brittain, Ed., Marcel Dekker, 1999 p.125.
8 D. Giron, Thermochim. Acta, 248 (1995) 1.
9 D. Giron, Labo-Pharma-Probl. Techn., 307 (1981) 151.
10 D. Giron, S.T.P. Pharma, 4 (1988) 330.
11 D. Giron, S.T.P. Pharma, 6 (1990) 87.
12 W. D. Walkling, W. R. Sisco, M. P. Newton, B. Y. Fegely, H. N. Plampin and F. A. Chrzanowski, Acta Pharm. Technol., 32 (1986) 10.
13 W. L. Rocco, Int. J. Pharm., 122 (1995) 17.
14 G. Zografi and E. Y. Shalaev, J. Pharm. Sci, 85 (1996) 1137.
15 Y. Takahashi, Drug Dev. Ind. Pharm., 11 (1985) 1543.
16 H. Kala, Pharmazie, 42 (1987) 524.
17 C. M. Adeyeye, Int. J. Pharm., 116 (1995) 65.
18 R. K. Kankari and D. J. W. Grant, Thermochim. Acta, 248 (1995) 61.
19 D. Giron, B. Edel and P. Piechon, Mol. Cryst. Liq. Cryst., 187 (1990) 297.
20 A. Burger, Acta Pharm. Technol. Suppl., 7 (1979) 107.
21 A. Burger and R. Ramberger, Mikrochim. Acta, (1979), II, 259.
22 A. Burger, Acta Pharm. Technol., 28 (1982) 17.
23 M. Soustelle, Handbook of Powder Technology, J. C. Williams and T. Allen, Ed., Vol. 9, Powder Technology and Pharmaceutical Processes, D. Chulia, M. Deleuil and Y. Pourcelot, Eds, Elsevier, 1994, p. 27.
24 J. Jacques, A. Collet and S. H. Wilen, Enantiomers, racemates and resolutions, J. Wiley, 1981.
25 D. Giron, Angewandte Chemische Thermodynamik und Thermoanalytic, Birkhäuser Verlag Basel, E. Marti and H. R. Oswald Ed., 1979, p. 227.
26 M. Kühnert-Brandstätter, (1971) Thermomicroscopy in the analysis of pharmaceuticals, Intern. Ser. Monog. Anal. Chem., Vol. 45 (R. Belcher and M. Freiser, Eds), Pergamon Press, Oxford and Thermomicroscopy of organic compounds in comprehensive Analytical Chemistry, Vol. XVI, G. Svehla ed., Elsevier, Amsterdam 1982, p.329.
27 P. M. Cooke, Chemical Microscopy, Anal. Chem., 68 (1996) 333R.
28 I. M. Vitez, A. W. Newman, M. Davidovich and C. Kiesnowski, Thermochim. Acta, 324 (1998) 187.
29 R. Kellner, M. Kühnert-Brandstätter and H. Malissa, Mikrochim. Acta, II (1988) 133.
30 R. J. Clark, Chem. Soc. Rev., 24 (1995) 187.
31 P. Valisa, Labo 2000, 11 (1997) 26.
32 M. Szelagiewicz, C. Marcolli, S. Cianferani, A. P. Hard, A. Vit, A. Burkhard, M. von Raumer, V. Ch. Hofmeier, A. Zilian, E. Francotte and R. Schenker, J. Therm. Anal. Cal., 57 (1999) 23.
33 E. Epple and H. K. Cammenga, Ber Bunsen-Ges. Phys. Chem., 96 (1992) 1774.
34 P. Conflant and A. M. Guyot-Hermann, Eur. J. Pharm. Biopharm., 40 (1994) 388.

35 D. Giron, M. Draghi, C. Goldbronn, S. Pfeffer and P. Piechon, J. Thermal Anal., 49 (1997) 913.
36 R. K. Cavatur and R. Suryanarayanan, Pharm. Dev. Technol., 3 (1998) 579.
37 K. Ashizawa, Netsu Sokutei, 25 (1998) 97.
38 T. Arii, A. Kishi and Y. Kobayashi, Thermochim. Acta, 325 (1999), 151, and Rigaku application note presented at the 48th annual Denver X-ray conference, 1999.
39 R. M. Caira, Top. Curr. Chem., 198 (1998) 163.
40 J. Masse, M. Bauer, P. Billot, M. Broquaire, A. Chauvet, J. Doveze, O. Garinot and D. Giron, S.T.P. Pharma Prat., 7 (1997) 235.
41 D. Giron, C. Goldbronn and P. Piechon, J. Pharm. Biom. Anal., 7 (1989) 1421.
42 D. Giron, P. Piechon, C. Goldbronn and S. Pfeffer, J. Therm. Anal. Cal., 57 (1999) 61.
43 D. Giron, PSST 1 (1998) 191 and 2 (1998) 262.
44 D. Giron, American Pharmaceutical Review, 3 (2000) 43.
45 A. Koda, S. Ito, S. Itai and K. Yamamoto, J. Pharm. Sci. Technol., Jpn., 60 (2000) 43.
46 D. Giron and C. Goldbronn, J. Thermal Anal., 49 (1997) 907 and J. Therm. Anal. Cal., 51 (1998) 727.
47 J. Han, G. Sangeeta and R. Suryanarayanan, Int. J. Pharm., 170 (1998) 63.
48 K. Byström, Thermometric Application Note 22004, 1990.
49 D. Giron, P. Remy, S. Thomas and E. Vilette, J. Thermal Anal., 48 (1997) 465.
50 K. Fiebich and M. Mutz, J. Therm. Anal. Cal., 57 (1999) 75.