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THERMAL ANALYSIS, MICROCALORIMETRY AND COMBINED TECHNIQUES FOR THE STUDY OF PHARMACEUTICALS*

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Abstract

Modern thermal analysis, microcalorimetry and new emerging combined techniques which deliver calorimetric, microscopic and spectroscopic data offer a powerful analytical battery for the study of pharmaceuticals. These techniques are very useful in all steps of development of new drug products as well as methods for quality control in production. The characterization of raw materials enables to understand the relationships between polymorphs, solvates and hydrates and to choose the proper development of new drug products with very small amount of material in a very short time. Information on stability, purity is valuable for new entities as well as for marketed drug substances from different suppliers. Excipients which vary from single organic or inorganic entity to complexes matrixes or polymers need to be characterized and properly controlled. The thermodynamic phase-diagrams are the basis of the studies of drug-excipients interactions. They are very useful for the development of new delivery systems. A great number of new formulations need proper knowledge of the behaviour of the glass transition temperature of the components. Semi-liquid systems, interactions in aqueous media are also successfully studied by these techniques.

Keywords: amorphous state, combined techniques, drug design, drug product development, drug substance, drug technology, DSC, excipients, failure investigations, hydrates, MDSC, microcalorimetry, pharmaceuticals, polymorphism, polymers, preformulation, process optimization, purity, quality control, solvates, stability, sub-ambient DSC, TG, temperature resolved X-ray diffraction, water interactions, thermal microscopy, water sorption-desorption

Introduction

Due to new mechanisms of actions, new molecules and new technologies pharmaceuticals play an important role in the improvement of health. Manufacturing pharmaceuticals imply generally two separated steps: manufacturing the active molecule (active ingredient or drug substance) and manufacturing the formulation

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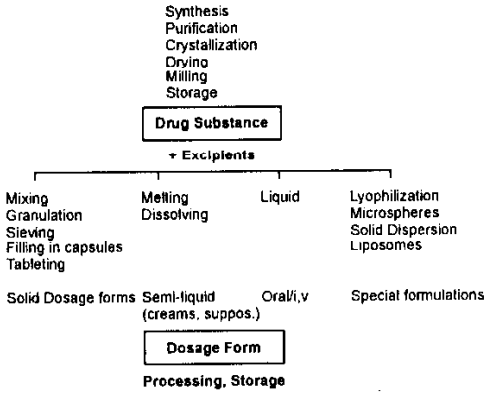

Fig. 1 Different steps leading to the drug product

(medicament or dosage form or drug product) which is given to the patient. The formulation itself plays a decisive role in the monitoring of the action in the body, e.g. quick action, long action, site of action. According to the medical needs, the medicament can be applied orally as a tablet, a capsule, a syrup, a solution, or intramusculary or parenterally as injection, as suppository, cream, gel, nasal spray or inhalation. Special delivery systems like depot forms, minipumps, patches allow constant delivery for patients without the needs to be in hospital. These formulations need auxiliary substances called excipients and adapted technologies for the manufacture of the drug product. For the patient it is mandatory that the product remains identical batch to batch and throughout its shelf-life in order to maintain the action at the desired time without unexpected side effects.

This is achieved by the proper control of the drug substance and of the excipients whatever their provenience as well as by the monitoring of the processing and the storage of the drug product. Figure 1 summarizes the main critical steps leading to the drug product.

Thermal analysis techniques have been used for pharmaceuticals for more than 30 years [1, 2], but the introduction of autosampler [3] the improvements of the instrumentation as well as the emerging of combined techniques highly increase the domains of applications and the robustness of the information delivered [4, 5].

Single compounds may be characterized through the measurements of heats and temperatures of specific heat, glass transition, melting, boiling, sublimation, decomposition, isomerization or heats of solution, water sorption-desorption.

For mixtures of several components, the phase diagram rules have to be considered. If there is no interaction between two compounds in the solid state and miscibility of the liquid state, an eutectic behavior will be observed during heating of the mixture. This behavior is the basis of the purity analysis by DSC. If there is no interaction in the solid state and no miscibility in the liquid state, the DSC scan of the mixture of two compounds will be the addition of the two DSC scans. This is the basis of quantitation of components in the drug product. An interaction in the solid state may result from the formation of solid-solution with partial or total miscibility,

Table 1 Main applications of thermal analysis and combined techniques in pharmaceutical development

Application	Techniques
Polymorphism	
Raw materials: characterization, control of crystallization, drying, milling	DSC, solution calorimetry, microcalorimetry, sub-ambient DSC, TG, DSC-spectroscopy, DSC-X-ray, Thermomicroscopy
Raw materials: storage conditions	DSC, TG, adsorption isotherms
Drug products: control of processes, granulation, mixing, milling, spray-drying, kneading, melting, lyophilization	DSC, TG, DSC-IR, DSC-Raman
Amorphous state	
Temperature T_g of glass transition of single components and influence of moisture, excipients	DSC, MDDSC
Study of polymers, copolymers	DSC, MDDSC
Optimization of formulations: microspheres, lyophilization, coating	DSC, MDDSC
Quantitation	DSC, microcalorimetry
Purity	
Raw materials: purification, stability	DSC
Stability	
Thermal decomposition, kinetics	DSC, TG, TG-MS, TG-IR
Compatibility	Microcalorimetry
Drug products	
Physical interactions, phase diagrams	DSC
Process optimization: solid dispersions, solid solutions, microspheres, modified release, lyophilisates	DSC, DSC-spectroscopy
Melting point of liquid formulations	Sub-ambient DSC
Identification, quantitation	DSC
Water interaction	
Gels, creams, polymers	DSC, sub-ambient DSC, DSC-microscopy
Determination bound water	DSC-TG, sub-ambient DSC
Liposomes	
Characterization hydrated phospholipid bilayer	DSC, microcalorimetry
Phospholipid–drug interactions	DSC, microcalorimetry

from the formation of complex, or from chemical reaction. In the case of hydrates or solvates the melting of these new compounds can be congruent or not.

Pharmaceuticals may also be studied in aqueous media in order to follow for example protein denaturation, gel formation, liposome formation or in order to design the conditions of lyophilization.

Table 1 summarizes the main applications of thermal analysis and combined techniques for pharmaceuticals.

The actual trends are the use of combined techniques (on line or not) and the introduction of modulated DSC (MDSC) for better studies of overlapping events. Studies may also be performed in moisture controlled atmospheres. Spectral data (IR, X-ray, Raman) are obtained at temperatures or at humidity levels where thermal events occur. Volatile compounds are identified by combining thermogravimetry with IR, MS, GC, Raman. Thermal microscopy is also combined with FT-IR and Raman allowing to reduce the amount of material observed. The improvement of the sensitivity and robustness of microcalorimetry allow a strong increase of the use of this technique for stability studies, quantitation of amorphous content, determination of heats of solution as well as for binding studies.

Like all analytical instruments, thermal analysis instruments offer automation. The instruments have to be installed , qualified and calibrated (IQ, OQ) and their performance periodically checked by the user (PQ) according to Isonorms and current GMP. Therefore pharmaceutical industry need certified reference standards, preferably organic substances with known melting points and melting enthalpies additionally to metals [4, 6].

Experimental

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

Calibrated Perkin Elmer DSC-7 with robot system, calibrated TGA 7 Perkin Elmer, calibrated instrument Scintag with autosampler or with heating cell for the X-ray diffraction experiments and a micro DSC II of Setaram for the microcalorimetric studies.

Polymorphism and pseudo-polymorphism

Polymorphism and pharmaceuticals

Polymorphism is the ability of a substance to crystallize into different crystalline states. The solid forms of the same compound are called polymorphs or crystalline modifications. Polymorphs show the same properties in the liquid or gaseous state but they behave differently in the solid state. The best known example of polymorphism is carbon, which can exist in the form of graphite or as a diamond. The amorphous state is characterized by crystallization in a non-ordered, random system, related to the liquid state.

The expression pseudo-polymorphism applies to hydrates and solvates as the result of compound formation with the solvent.

A recent overview upon thermal analysis and calorimetric methods in the characterization of polymorphs and solvates references more than 300 active ingredients [7].

Table 2 summarizes the consequences of polymorphism and pseudo-polymorphism in pharmaceutical area. The main impact of polymorphism is the change of activity and toxicity. This behaviour is so important for pharmaceuticals that the International Conference of Harmonization (ICH) requires a study of the polymorphism of each new entity before its introduction on the market [8]. The process of transformation of one polymorph into another one is a phase transition, which may also occur during storage or during processing. If the phase transition is reversible, the two polymorphs are enantiotrops. The energy of the transition by heating is endothermic. If the phase transition is irreversible, the two polymorphs are monotrops. Only one form is stable whatever the temperature and the transformation of the metastable form to the stable one is exothermic. For kinetic reasons metastable forms may exist outside phase diagrams equilibrium curves.

Both thermodynamic and kinetic aspects have to be considered. The thermodynamic one is the driving force, the kinetic one allows to understand failures and unexpected phenomena; In organic chemistry, metastable forms may survive years unless for kinetic reasons, the influence of moisture, catalysts, impurities, excipients, temperature, the transformation into the stable form occurs spontaneously. In the last step of synthesis, the temperature is generally lowered during crystallization. At the beginning of the crystallization the more soluble form, metastable, crystallizes. Through solvent mediated transition the stable form should be obtained. But depending on the crystal growth and the solubility, this transformation may not happen and the metastable form is obtained. In the case of enantiotropy, the stable form is different above and below the reversible equilibrium temperature. The same observation applies above or below the transition of the solvated form into the desolvated one. 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 forms obtained during drying [9].

The same remarks go for the manufacturing processes of the drug product which involve the use of solvents or melting (suppositories, solid dispersions; creams, granulation; liquid formulations, microspheres). In a wet granulation we could identify the monohydrate of phenobarbital [10].

Table 2 Consequences of polymorphism and pseudo-polymorphism

Property	Area concerned
Solubility, dissolution rate	Activity, toxicity, solubility, drug product
Chemical stability	Drug substance, drug product
Purification	Yield, drug substance
Processability	Drug substance, drug product
Process robustness	Drug substance, drug product
Hygroscopicity	Homogeneity drug substance, drug product
Hygroscopicity	Stability drug substance, drug product
Physical change during storage	Drug substance, excipient, drug product
Wrong results of preformulation	Drug substance, drug product

Interpretations

The thermodynamic information is the best obtained from DSC (melting point, melting energy) or from solution calorimetry (comparison of the heat of solutions) by using the Burger's rules [11]. Furthermore, the forms may be obtained in situ in DSC or in moisture controlled chambers. The example given in Fig. 2 illustrates the case of enantiotropic or monotropic transitions with the influence of the kinetic. Figure 2a shows the DSC scan of the first sample of a drug substance, called polymorph A. At 10 K min^{-1} an exotherm immediately follows the melting of the form A and a form B is obtained. This dual melting shows that some kinetic factors hinder a solid–solid transformation (endothermic for enantiotropy or exothermic for monotropy) which should be observed. Form B crystallizes spontaneously from the melt since B is stable in this temperature range. It was possible to obtain this form B in situ and to scan the DSC curve of the form B. The polymorphic study of this compound leads to obtain a third form C. Neither A nor B show a transformation in solid state (DSC) into C. Quick heating enabled us to calculate the heat of melting of A. The temperature and heat of melting are: Form A: 101°C, $\Delta H \geq 81$ J g^{-1}, Form B: 119°C, ΔH=78 J g^{-1}, Form C: 121°C, ΔH=108 J g^{-1}. According to the Burger's rule C is the stable form, A and B are enantiotrops and both monotrops to C. In suspension with solvents, the transformation of A or B into C is strongly accelerated by the presence of C. The DSC scan of a mixture 1:1 of A and C is given in Fig. 2b. The heat of melting of C is quite higher than expected if no transformation of the other forms would occur, resulting of the presence of seeds of the modification C.

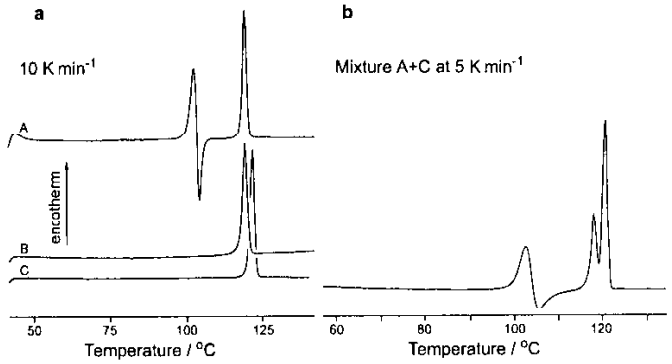

Fig. 2 Example of kinetic behaviour of enantiotropic and monotropic transformations. A and B are monotrops to C; a – DSC at 10 K min^{-1} A: Form A, B: Form B, C: Form C; b – DSC at 5 K min^{-1} of a mixture 1:1 of forms A and C

Thermal events may overlap or interpretation may be difficult. Combined techniques now mostly offered by instrument manufacturers are very efficient as demonstrated by the two following examples. Figure 3 deals with tetracaine hydrochloride. This drug substance is described in the European pharmacopea (Ph. Eur.) with two polymorphs. The study of this substance enabled us to identify six crystalline modifications, an amorphous form, a tetrahydrate, a monohydrate and a hemi-hydrate

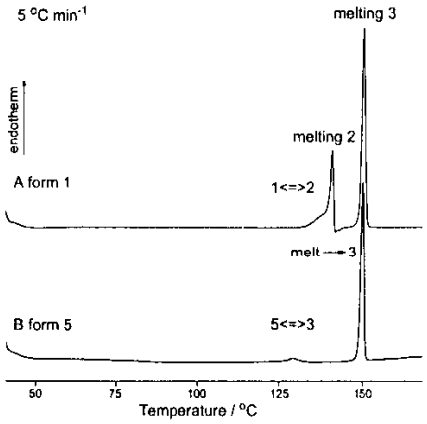

Fig. 3 Tetracaine hydrochloride. DSC scans of two crystalline modifications; A: form 1, B: form 5

[12]. Our study included temperature resolved X-ray diffraction. Figure 3 shows the DSC scans of the form 1 and the form 5. The DSC curve of the form 5 shows a classical endothermic enantiotropic transition of form 5 into the high melting form 3. The relationship between the two modifications 1 and 3 described in the pharmacopea was very interesting. The DSC scan given in Fig. 3 shows that two events overlap. Our study with temperature resolved X-ray diffraction demonstrated that form 1 transforms into form 2 prior to the transition to the high melting form 3.

Figure 4a is one experiment which demonstrate that form 1 transforms reversibly into form 2 (1↔2) and Fig. 4b shows the transformation 1→2→3. The melting of forms 1 and 2 may overlap with the transformation 1↔2 depending on the heating rate. With DSC alone it would not be possible to identify the intermediate form 2.

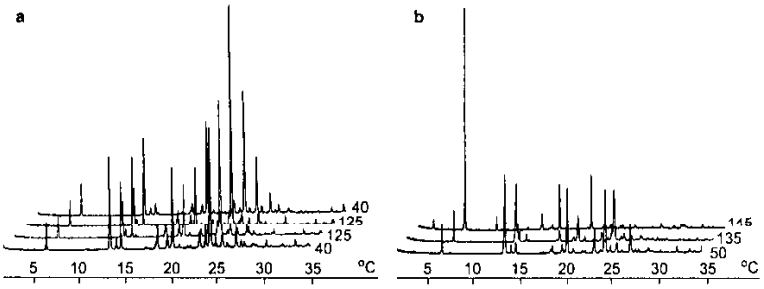

Fig. 4 Tetracaine hydrochloride; Examples of temperature resolved X-ray diffraction study of the form 1. a – Heating from 40 to 125° C and cooling back to 40° C. Reversibility of the transition; b – Heating from 40 to 145° C. Successive transformations 1→2→3

Figure 5 shows the usefulness of combined technique in order to avoid wrong interpretation. The DSC curve of the drug substance which is a malonate salt is very similar to a dual melting of two polymorphs. The TG curve shows a loss of mass of

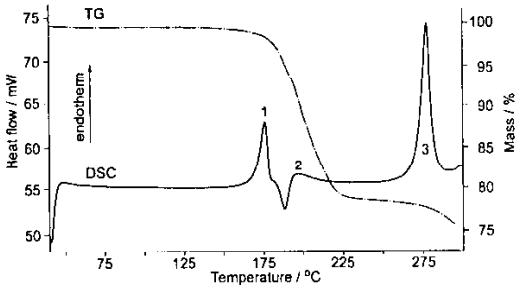

Fig. 5 Interpretation of DSC curves. Use of combined methods: DSC and TG curves of a malonate salt; 1 – melting of the salt; 2 – decomposition of malonic acid and evolving of CO_2; 3 – melting of the base of the drug substance. TG: 21.5% loss of mass; theoretical amount of malonic acid: 21.1%

21.5% just after the endo-exothermic peaks. The evolved compound is CO_2 as detectable by IR, the compound obtained is the base of the drug substance as followed by X-ray diffraction. FT-IR microscopy with a heating cell can confirm the finding. In this case the base of the drug substance is formed and is responsible for the second melting endothermic peak. In the case of aspartame, TG-IR [5] as well as TG-MS [13] could detect the loss of methanol. The last melting peak was the melting peak of the degradation product (3-carboxymethyl 6 benzyl 2,5 dioxopiperazine) [14].

Water sorption–desorption isotherms

Moisture is a component of the atmosphere. International companies have to take into account all climatic conditions. In Japan or in tropical climates high moisture levels of >75% *r.h.* may be attained. In hot climates this can be reduced to less than 40% *r.h.*

Water adsorption and desorption isotherms of drug substances and excipients need to be known, especially if changes of crystalline modifications occur. At each temperature corresponds a critical value of the relative humidity (*r.h.*) at which loss or uptake of bound water occurs. For instance, Medetromidine is anhydrous only if the *r.h.* is less than 30% at ambient temperature [15]. Thermodynamic aspects of moisture sorption isotherms have been recently analyzed by Sacchetti [16].

There are often different behaviours in sorption and desorption as illustrated in the example of Fig. 6. The example of formation of theophylline monohydrate at the surface of tablets has been intensively studied [17, 18]. In the case of the formation of several hydrates, the kinetic of the dehydration may be different to the hydration. It is the case of Nafragel hydrochloride [19]. The sorption of water occurs in two steps via the hemihydrate. The monohydrate formed loses water in one step into the anhydrous form. Due to different kinetics, the anhydrous form, the hemi-hydrate and the monohydrate coexist. Polymorphs behave differently as demonstrated in Fig. 6. Even one metastable form may be transformed into the hydrated form in solid state and not the stable one [7]. Hydrates have also polymorphs [7]. Solvates may transform into hydrates. In certain cases even water is not tightly bound and difficult to be characterized in DSC. X-ray diffraction cells with variable humidities are state of the art for such studies. Sub-ambient DSC is also a good way to calculate bound

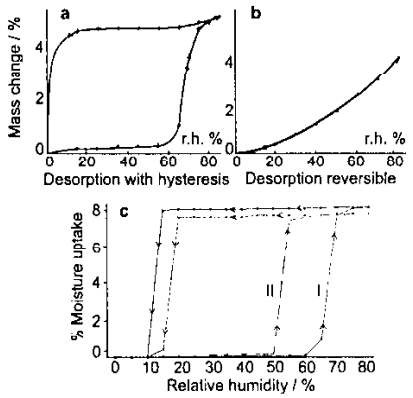

Fig. 6 Examples of water sorption-desorption isotherms; a – desorption with hysteresis; b – desorption reversible; c – Hydrate formation of two polymorphs. The metastable form 2 take up water at lower *r.h.* than the stable one

water and to characterize not tightly bound hydrates [20]. Instruments allow to plot the water sorption and desorption isotherms gravimetrically. Microcalorimetry can also be used for such studies.

From ordered state to disordered state

Amorphous state is produced by fast crystallizations, precipitations, drying, milling, freeze drying or quick cooling from the melt. Often there is a memory of the crystalline state, especially after drying of solvated forms. Amorphous substances are generally hygroscopic, have a better solubility and bioavailability; they have good tabletting properties but are difficult to be milled. Very often they are chemically less stable. They tend to transform into crystalline forms upon storage. The transitions are generally fast at temperatures above the glass transition. The temperature of the glass transition is depressed by water. When the drug substance exhibit different crystalline modifications, the amorphous form may transform into one or the other depending on the temperature and the humidity, as it is the case of indomethacine [21]. Figure 7 shows the DSC scans of a crystalline drug substance and an amorphous sample. The enthalpy of the exothermic crystallization has been suggested for quantitation purposes. For a drug substance we could detect easily less than 5% amorphous form [6]. In pharmaceutical area quantitation of the amorphous part is generally carried out by X-ray diffraction. The limit of the method lying around 5–10%, the new method using microcalorimetry has been found very attractive [22–25]. The exothermic crystallization is determined isothermally. The temperature and the duration of the experience are designed by the humidity level of the experiment chosen since water depresses the glass transition temperature and consequently the temperature of crystallization.

Figure 8 shows the microcalorimetric heat flow curve of a sample spiked with 6% amorphous form and the linear relation calculated between the signal output and

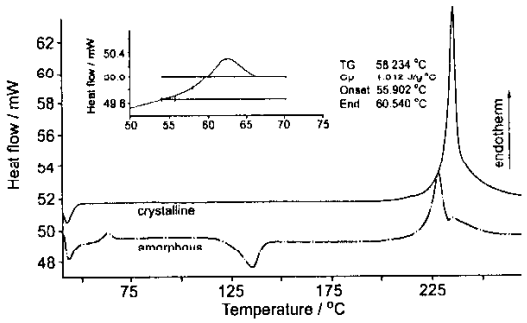

Fig. 7 DSC curves of crystalline and amorphous samples of a hydrochloride salt of a drug substance

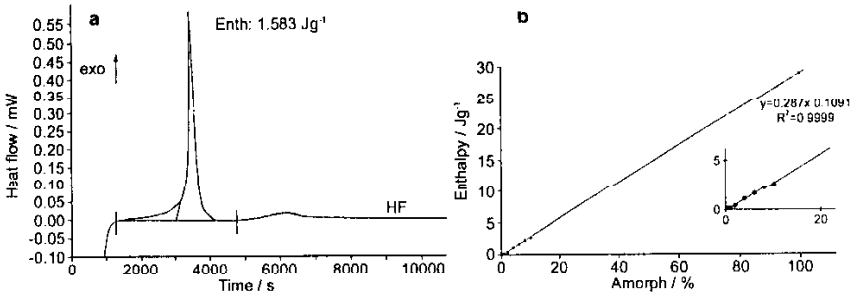

Fig. 8 Example of microcalorimetric determination of the amorphous content of a drug substance at 30°C and 75% *r.h.*; a – microcalorimetric output for a sample containing 6% amorphous form; b – linear relationship between the heat flow and the amorphous content (r^2=0.9999)

the amount of amorphous form [25]. In this technique different aspects like transformation into different crystalline forms, desorption energies of the adsorbed water are also to be considered. Solution calorimetry is also used for quantitation [7, 26].

Purity determination

The purity determination by DSC is based on the van t'Hoff law for dilute solutions (purity ≥98%). The eutectic impurities are determined together in mole % without the need of reference standard of the drug substance. This method often considered as absolute purity method is described in the US pharmacopea (USP XXIII). Details about optimization and validation of DSC purity determination have been recently discussed [27]. Automation makes the method very attractive since results are obtained in less than 30 min with only 1–2 mg or less and the sum of impurities is determined. Since for safety reasons, each impurity of the drug substance has to be individually quantified, this technique cannot replace separation techniques for the drug substance, but add a great value of information. Raw materials, drug substance

and excipients are worldwide distributed and the origin may change the quality. The best example we had is the case of β-hydroxypropyltheophylline for which DSC as well as Phase Solubility Analysis (PSA) were able to determine impurities from 0% up to 5% in the batches from different suppliers. The chromatographic purity method was unable to distinguish the quality of the batches [28]. Homogeneity of batches, stability screening, comparison of stability behaviour of batches, choice of proper storage conditions, re-testing are valuable applications. In the same scan the melting point can be determined [29]. Radiolabelled substances can be also analyzed [6]. The following two examples demonstrate the value of this method for quality control. Figure 9 shows the DSC scans of a sample containing 1.5% unknown impurity not detected and the sample purified in a mixture acetone/hexane 1:1. Figure 10 illustrates the use of the technique for a substance with different synthetic pathways. One or several impurities were not detected in the new synthetic pathway.

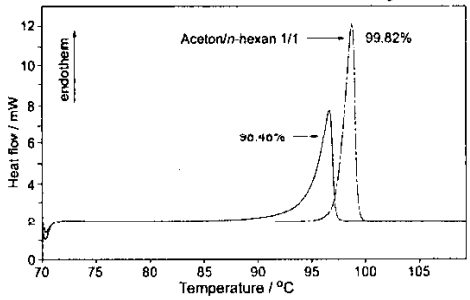

Fig. 9 DSC as monitoring of purification. The initial sample has a purity of 98.48%. The purity of the sample purified in a mixture acetone/hexane 1:1 is 99.8%

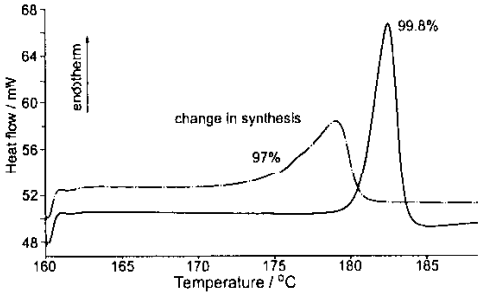

Fig. 10 Use of DSC for checking a new synthesis process. Current process purity: 99.8%; New synthesis: 97%

In the case of enantiomeric impurities, the type of the phase diagram (conglomerate, true racemate, pseudo-racemates) is relevant for the determination of purity or for the monitoring of purification. Purification of intermediates or of drug substance via conglomerates or via enrichment or via salts are based on the knowledge of phase diagrams by DSC [30, 4].

Polymers

Polymers widely used for pharmaceuticals as a part of the drug product, (excipients) or as packaging materials are studied by various thermal analysis techniques [31]. The number of polymeric excipients is very high. Cellulose derivatives, gela-

Table 3 Glass transitions of some polymers used as excipient

Polymer	Glass transition (T_g/°C)
Polyvinylpyrrolidone	
Kollidon 12	93
Kollidon 17	130
Kollidon 25	155
Kollidon 30	168
Kollidon 90	178
Crospovidone	185
Ethylcellulose N20	120
Ethylcellulose N50	126
Ethylcellulose N100	128
Methylcellulose: Methocel A15	176
Hydroxypropoxymethylcellulose	
Methocel E5	137
Methocel E15	156
Methocel E4M	164
Methocel K15M	180
Methocel K100M	180
Hydroxypropylmethylcellulosephthalate	
HP 50	140
HP 55	134
Polyvinylacetate	
Mowilith 50	65
Vinnapas B 1.5	44
Propyleneglycol	
PPG-1000	199
PPG-2000	201
PPG-4000	201

tine, starch derivatives and polyvinylpyrrolidons commonly used. Crystallinity and molecular mass are relevant for the targeted application. Tablet production containing amorphous polymers should be done at lower temperatures than the glass transition in order to obtain material with minimum porosity [32]. Gelatinization of starch derivatives allows their characterization. Blends of biodegradable polymers and copolymers of e.g. polylactic acid, polyglycolic acid, polyethylenecarbonate are also used for special formulations like microspheres for subcutaneous injections. Table 3 gives some glass transition temperatures of classical amorphous polymeric excipients. Polyethyleneglycols can be liquid or solid. The melting point depends on the molecular mass [2]. Both once folded chain or extended chain crystals may coexist [33]. Water also depresses the melting point of polyethyleneglycols [4]. A high varieties of polymers derived from fatty acids are used in liquid and semi-liquid formulations. These polymeric excipients undergo polymorphic changes during storage, typical for fatty acid derivatives.

For packaging materials the use of DSC is recommended for the characterization of the melting of different qualities of polyethylene by the US pharmacopea. Crystallinity of polyethylene has been studied [34]. Film coated tablets are generally obtained by spraying polymer or mixtures in solution or in suspension in aqueous medium. Coating and drying should be carried out below the glass transition. Thermal analysis techniques give information regarding interactions of the polymer with plasticizers or additives and compatibility of blends.

Interactions of water with polymers are important for modified release formulations for which the swelling or gel formation of the polymer in gastric fluid is relevant for the dissolution profile of the active ingredient. Bound water can also act negatively for degradation processes when changes of temperature deliver unbounded water. A high amount of water can be bound to the amorphous polymer, resulting in a dramatic decrease of the temperature of the glass transition, for example for hydroxypropylmethylcellulose HPMC 4000, we observed a decrease of approx. 80°C [4]. Thermogravimetry combined or not with GC or IR and subambient DSC are very useful for the determination of residual solvents or for the study of water interactions.

Drug products

Solid drug products

DSC curves are not adequate as chemical compatibility screening since physical interactions are observed [4, 28]. Furthermore the thermal events are observed at temperatures for which no moisture remain in the system studied. But DSC curves after storage are very valuable for the understanding of physical and chemical changes. The knowledge of the phase diagrams is of great value and is used for the optimization of the formulations. For example in solid dispersions with polyethyleneglycols, the drug substance is finely dispersed in the polyethyleneglycol and changes of dissolution profile are obtained. Figure 11 shows the diagram obtained between darodipine and polyethyleneglycol. These solid dispersions being often obtained by melting the precise knowledge of the phase diagram allows to validate the manufacturing process.

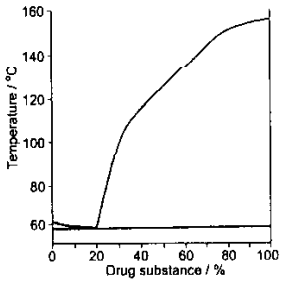

Fig. 11 Phase diagram between polyethyleneglycol 6000 and darodipine. The temperature of melt is fixed according to the phase diagram

In case of solid solutions, e.g. with polyvinylpyrrolidines, the drug substance should remain amorphous. The loading is currently checked by DSC and X-ray diffraction [4].

Complex formation with cyclodextrins are currently analyzed by DSC. Often no complex is formed in solid state and the complexation occurs during dissolution in the body.

For microspheres, interactions with the polymer (study of the glass transition) as well as the crystallinity of the drug substance are studied by thermal techniques.

If the components of the formulations do not show any interaction, it is possible to identify and quantify the components. Figure 12 deals with a drug substance formulated with mannitol and polyethyleneglycol. Amorphous excipient does not interfere in the DSC. The formation of an eutectic between the drug substance and mannitol is obvious. Polyethyleneglycol does not interact and can be quantified. For an-

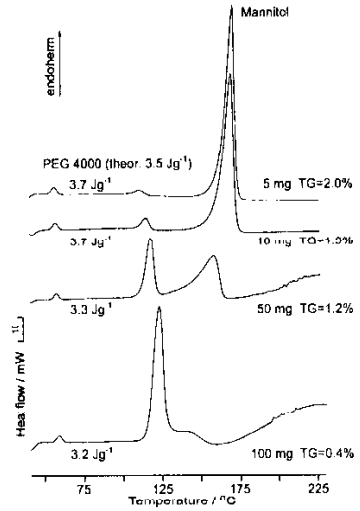

Fig. 12 DSC curve of capsules of different strengths (5, 10, 50, 100 mg) of a drug substance with the same composition. The excipient mannitol and the drug substance have an eutectic behaviour. Polyethylenglycol does not interact and can be determined in the capsules

Table 4 Example of assay determination of drug substance or excipient in tablets or capsules

Product		Active ingredient	% of theoretical value
Capsule		Drug substance	98.0 (n=10)
Capsule		Mannitol	97.1 (n=1)
Capsule	10 mg 50 mg	Drug substance in development	98.2 (n=1) 100.0 (n=1)
Doliprane tablet		Paracetamol	101.0 (n=1)
Haldol tablet		Haloperidol	94.0 (n=10)
Pellet batch 1		Saccharose	100.1 (n=1)
Visken tablet		Pindolol	98.0 (n=1)

other formulation we were able to identify poloxamer, polyethyleneglycol, glycerol-monostearate and lactose monohydrate. DSC was also used for the determination of the content uniformity of Cutina (hydrogenated castor oil) [35]. Table 4 shows some examples of quantitative determination of components of the drug product. Polymorphic changes of the drug substance in tablets or capsules can be also studied [4].

Liquid, semi-liquid formulations

Suppositories, microemulsions, solutions in soft gelatine capsules, creams are formulations for which the behaviour of the excipient is predominant. The development of the formulation is strongly based on the quality and on the polymorphic behaviour of the excipient. DSC and sub-ambient DSC are the best method for the development and for quality control of the excipient as well as for the formulation [36, 37, 38]. Figure 13 shows the melting behaviour of different excipients used for suppositories. The melting point of the excipient may be highly relevant for the dissolution of the drug substance, if the delivery is done only after melting of the excipient [4, 37]. Since climatic conditions give rise to changes of temperature, a liquid formulation should remain liquid for the user. DSC is very helpful for the fractionation of glyceride derivatives [4]. Figure 14 shows the critical domain of the DSC curves of different batches of a liquid excipient.

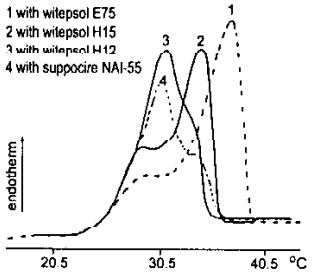

Fig. 13 DSC melting curves of some excipients used as suppository masses

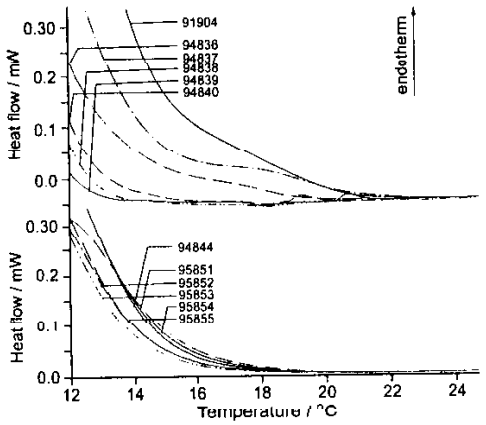

Fig. 14 Quality control of a liquid glyceride-corn oil excipient. Examination of the end of melting in the critical range 15–25° C

Interaction with water

The phase diagrams of drug substance and excipients with water as well as the study of the temperature of glass transition are the basis of the choice of the condi-

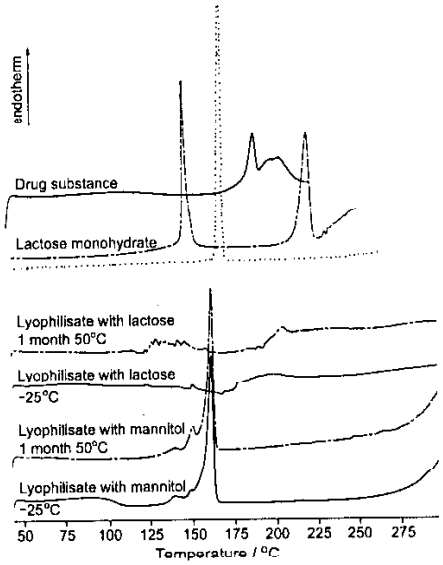

Fig. 15 DSC curves of two formulations of lyophilisates of a drug substance. The drug substance remain amorphous. Lactose is also amorphous. Mannitol has different polymorphs

tions of freeze dried or spray dried formulations [39–42]. The lyophilisates are well characterized by DSC, TG and combined techniques as demonstrated in Fig. 15. In this example, after lyophilization the drug substance and lactose remain amorphous and mannitol exhibit different polymorphs.

The polymorphic behaviour of mannitol during lyophilization has been recently studied [43].

For proteins, it is suitable to have excipients in the formulation which remain amorphous. Tetralose was found to be a very efficient lyoprotectant [44].

Freezable water is determined by the measurement of the melting peak of ice as demonstrated in Fig. 16 for Methocel K15M, Cetylpalmitate and a gel. This technique is currently applied for gels and creams [45].

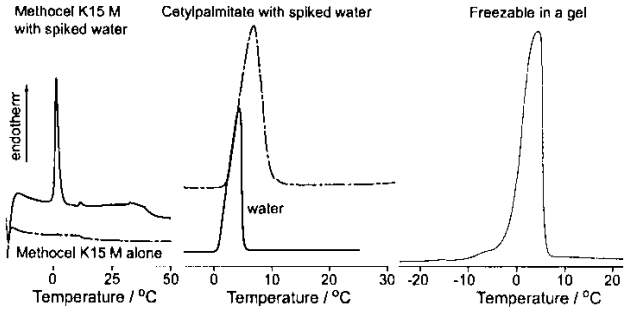

Fig. 16 Measurement of freezable water in a polymeric excipient, hydroxypropylmethylcellulose Methocel K15 M, in cetylpalmitate and in a gel drug product

Clausse *et al.* studied the water transfer within water oil (W/O) emulsions [46] and underlined the use of DSC in the prediction of stability of emulsions [47].

Liposomes are multilayered vesicles consisting of concentric bilayers of phospholipids interdispersed with aqueous phases. In aqueous media, the phospholipids undergo gel, liquid crystalline transitions easy to detect by DSC. The study of the change of these transitions, temperature, peak width and energy allows to characterize the hydrated phospholipid bilayers and to study the liposome formation with drugs [48].

The study of the denaturation of proteins is also an area of application of DSC and microcalorimetry [49]. A review of biological applications has been published by Collet and Brown [50] recently.

Stability and failure investigations

The stability of drug substance, excipients and drug products through the time of application is mandatory. Purity determination by DSC after storage of raw materials [27] and even of the drug product [35] is a typical application. In the beginning of development it is suitable to have methodologies for prediction of stability. Due to the increase of sensitivity microcalorimetry is increasingly used since results are obtained isothermally at temperatures relevant for the applications [51]. Figure 17

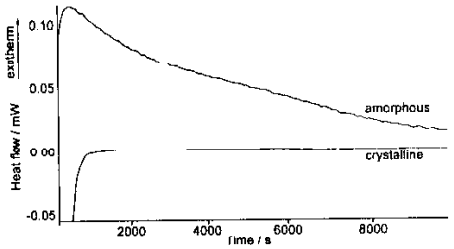

Fig. 17 Microcalorimetric study of the stability behaviour of the crystalline and the amorphous forms of a drug substance at 30°C/100% *r.h.*

shows the microcalorimetric outputs for a crystalline sample and an amorphous sample of a drug substance.

Physical stability is also a concern of pharmaceuticals: loss of activity through polymorphic change, precipitation in liquid formulations due to polymorphic changes of drug or of excipients, dissociation of salt.

The physical instability of fatty acid derivatives is well known. Glycerides derivatives are generally obtained in metastable state and transform to the stable form on aging. This results in an increase of the melting point. In a suppository formulation containing paracetamol, we could determine an increase of 3°C of the melting point after storage 18 months at 25°C. Only 50% of the Witepsol was melted at 37°C! In another case we compared the stability behaviour of two excipients for a modified release form. Precirol undergoes a strong change during storage but cetylpalmitate [4] does not.

DSC and combined techniques (FT-IR, Raman, X-ray) are very helpful in failure investigations as demonstrated in the last example [5]. The drug substance was a hy-

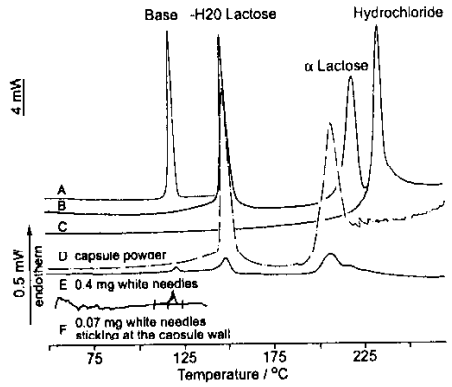

Fig. 18 Failure investigations: DSC study of needles appearing, sticking in the gelatine internal walls of a capsule formulation of a drug substance hydrochloride. Lactose monohydrate is the excipient; A – Drug substance as base; B – lactose monohydrate; C – Drug substance hydrochloride; D – capsule powder; E – and F – needles of the capsule after storage in high humidity; F – is mainly the drug substance base alone

drochloride salt. The formulation was a hard gelatine capsule filled with a mixture drug substance–lactose monohydrate. Stability screenings were performed with mixtures of drug substance and lactose. During stability studies of this capsule formulation, the formation of needles sticking at the walls of the capsule was observed. Gelatine capsules contain a high amount of water. The needles were the base of the drug substance resulting from the dissociation of the hydrochloride in presence of gelatine and water as demonstrated by DSC and FT-IR. The DSC study of this phenomenon is summarized by the curves given in Fig. 18. The DSC curves of the drug substance as base (A), of the lactose monohydrate (B), of the drug substance as hydrochloride (C) can be compared with the DSC curves of the capsule powder (D) and with two samples of needles isolated from the wall of the capsule. The hydrochloride of the drug substance has a physical interaction with lactose (displacement of the melting peaks of the drug substance as hydrochloride and of the α-lactose, curve D). The needles are more or less pure drug substance as base and the melting peak of base could be used for quantitation purposes.

Conclusion

This overview cannot cover all applications of thermal techniques in pharmaceutical area. We aim at showing how the improvement of the thermal analysis current techniques regarding qualification, reproducibility, automation as well as the new emerging combined techniques and the very sensitive microcalorimetry will increase their use for faster development and for safe drug products.

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