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Applications of thermoanalytical techniques for studying polymorphism and solid-state transformations in pharmaceutical industry

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ABSTRACT

Knowledge of the polymorphic behavior of a drug is an essential requirement of drug formulation development in terms of its dissolution, bioavailability, stability and to a great extent the regulatory considerations. Polymorphic forms exhibiting different lattice structures tend to melt at different temperatures and therefore differential scanning calorimetry can be a unique tool in detecting these forms. The widespread uses of DSC within pharmaceutical research include polymorphism, pseudopolymorphism and glassy systems. The remarkable versatility of this technique is that the method essentially measures heat flow processes that are applicable to all systems and hence characterization of polymorphs with a far greater degree of sophistication. In developing a particular dosage form, there is a considerable interest in optimizing the removal of residual organic solvents. Understanding the nature of the binding of the volatile component and assessing the kinetics of the weight loss process, it is possible to get information on longer term stability. Thermogravimetric analysis along with DSC and X-ray diffraction techniques can be useful to obtain a more detailed analysis of the kinetics of desolvation processes such as dehydration and the nature of the drug-excipient or excipient-solvent interaction. More effective removal of solvent can be ascertained with appropriate kinetic analysis of loss process and TGA can be very useful in finalizing design of manufacturing protocols. This review gives representative examples of the widespread use of DSC and TGA to characterize polymorphs, solvates and investigate amorphous states. Examples outlined here envisage the thermodynamic parameters associated with polymorphic transitions and assessment of the relaxation behavior of amorphous pharmaceutical materials.

KEYWORDS

Polymorphism;
Enantiotropy;
Monotropy;
Active pharmaceutical ingredient (API);
Excipient (placebo);
Differential scanning calorimetry (DSC);
Thermogravimetric analysis (TGA);
Hot stage microscopy.

INTRODUCTION

Different crystal packing arrangements of the same molecular species in solid-state is termed as polymorphism[1]. Pseudopolymorphism or solvatomorphic systems[2] are the solid state modifications of the active pharmaceutical ingredients or excipient whereby the solvent is incorporated into the crystal lattice of the par-

ent compound. In case of pharmaceutical systems, the crystal form is termed as hydrate if the solvent is water. Solvates in comparison to anhydrous form can differ to a great extent in their physical and biological behavior[3]. Since physical and chemical property are different in case of crystal structures, any technique that measures properties of these forms may in principle be used to detect polymorphism. Most of the time such differences are marginally so, and therefore it is important to look at potentially polymorphic systems by a variety of techniques. It is necessary to consider and understand the sensitivity of the technique which differentiates crystal structure or molecular environment of the polymorph contrary to its molecular structure. Most commonly used techniques for polymorphic screening are XRD, FT-IR, FT-Raman DSC, TGA and thermomicroscopy[4]. Although the extent to which polymorphic behavior of a drug substance or an excipient may be predicted is limited, the characterization of polymorphs remains a highly important aspect of pharmaceutical sciences. The real benefit of DSC studies over other techniques like X-ray diffraction lies in more advanced thermodynamic analysis of polymorphs, especially in differentiating the monotropic or enantiotropic systems[5]. Identifying the transition temperature of the enantiotropic polymorphs can be best achieved by DSC[6].

X-ray powder crystallographic methods indicate the crystal structure differences and are useful in defining the polymorphic system[7]. In principle single crystal X-ray diffraction method[8] is used for the determination of detailed molecular and crystal structure, for example bond lengths, bond angles etc. X-ray powder diffraction methods are often used for the polymorphic screening of individual polymorphic forms, evaluation of degree of crystallinity and quantification of one polymorphic form in another[9]. Nowadays, single crystal structure solution when applied to powder data followed by Rietveld refinement methods[10], difficult and larger crystal structure problems can be solved. Quantitative X-ray powder diffraction technique is based on the assumption that the integrated intensity of a diffraction peak is proportional to the amount of polymorph present. The ideal arrangement of individual crystallites in the sample specimen should be completely random but when these crystallites take up some particular orientation due to their non-spherical habits, sample is said to have preferred orientation[11]. This effect influences the intensities of the powder diffraction lines and hence the quantification methods.

Solid-state nuclear magnetic resonance spectroscopy (SS-NMR)[12] provides information on the environment of magnetically active individual atoms. Polymorphs with varying crystalline structures possess some atoms with different crystal environment. Change in the environment of the atoms can be due to conformational differences in different polymorphs. The use of high power proton decoupling, magic-angle spinning and cross-polarization leads to high resolution spectra and structural information can be extracted for polymorphic evaluation[13]. SS-NMR offers a number of advantages. Particle size does not influence the SS-NMR signals. The intensity of the signal is directly proportional to the number of nuclei producing it. Therefore, mixtures of polymorphic form can be recognized. Although the sample preparation is not an issue, during the measurements, the high spinning rates required for magic angle spinning technique (MAS) generate mechanical stress and local heating may result in polymorphic transformations. Structural and molecular conformations in the polymorphs can be investigated with infra-red[14] and Raman spectroscopy[15].

There are certain examples available in literature which advocates the use of DSC in pharmaceutical industry besides the use of equally important supplementary techniques. In thermoanalytical techniques an important physical property is determined as a function of externally applied temperature. The phenomena or reactions essentially monitored in these techniques can be endothermic in nature which includes melting, boiling, sublimation, vaporization, desolvation, chemical decomposition etc. or exothermic in nature which includes crystallization, oxidative decomposition etc. In pre-formulation studies, thermoanalytical methods can be used to evaluate the polymorphism, pseudopolymorphism, degradation studies, drug-excipient compatibility and other important desirable qualities[16].

ORIGIN OF POLYMORPHISM

The origin of the polymorphism lies in the different inter- and intramolecular interactions. These non-covalent interactions like hydrogen bonding, van der

Waals interactions, π-π stacking and electrostatic interactions, provide strength and directionality in crystal structure. Due to these interactions, a variety of unit cell structure exists and thence different polymorphs. Different polymorphs or 'forms' have different free energies and hence display different physical properties such as melting point, density, etc. and to some extent the chemical stability.

PSEUDOPOLYMORPHS OR SOLVATES

Pharmaceutical solvates and hydrates have been studied in great detail. Differentiation between surface water and water of crystallization may not always be easy. Same is the case with a true solvate and sorbed solvent. The desolvation of a material can result in the formation of crystal lattice unchanged compared to the solvate or a mixture of crystalline and amorphous state or a dehydrated form completely different from the original lattice. Assuming that there is no decomposition taking place prior to melting, a typical DSC thermogram of a hydrate may consist of an endothermic peak corresponding to loss of water and an endotherm at higher temperature corresponding to melting of an anhydrous form. The loss of water molecule may take place well above 100°C. It is also possible that solvent is lost at or above the melting point and in this case anhydrous form recrystallizes from melt or an amorphous form indicated by an exotherm. There might be cases, where one can not deny the possibility of solvate exhibiting polymorphism, i.e., the different polymorphs in which the solvate can exist.

TABLE 1 : An overview of initial investigations on raw material to establish initial form and related properties

Crystal structure	Determine crystal structure; useful for subsequent identification of forms
XRPD	Obtain reference powder data for known forms; confirm single crystal structure is representative of polycrystalline sample
Raman/FT-IR	Obtain reference data/fingerprints
DSC/TGA	Determine melting points and identify transformations
VT-XRPD	Structural characterization of polycrystalline phases obtained from thermal transformations in situ using SDPD
Dynamic vapor sorption/slurrying	Assess hydrate/solvate formation
Solubility/dissolution	Characterize form and assess solution meditated transformations

Abbreviations : DSC, differential scanning calorimetry; FT-IR, Fourier transform-infrared spectroscopy; SDPD, structure determination from powder data methods; TGA, thermogravimetric analysis; VT-XRPD, variable temperature X-ray powder diffraction; XRPD, X-ray powder diffraction.

Experimental conditions and experimental parameters should also be considered before concluding any remark about the behavior of hydrates or solvates. DSC measurements with hermetically sealed pans lead to suppression of water loss peaks because buildup of headspace pressure affects the dehydration process. Water loss peak clarity can be achieved by using open pans or pin-holed pans. By using commercially available pin-holed pans reproducibility in evaporation process can be achieved. It is also important to consider the particle size of the material under investigation. Flow rate of purge gas also influences the melting behavior of the hydrates as it affects efficiency of removal of water from hydrates and thermal conductivity of the purged cell. Effects of heating rate on the DSC thermograms have also been investigated.

METHODOLOGY USED IN DSC

Differential scanning calorimetry is an easier to use thermoanalytical technique which can be routinely used for both qualitative as well as quantitative purpose. In this technique, the sample and reference materials are maintained at the same temperature and to keep the same temperature, the required heat flow is measured. DSC plots obtained are termed as DSC thermograms and represent differential heating rate i.e., J/s or cal/s on ordinate and temperature on abscissa. Integrated peak areas indicate the heat absorbed or evolved by the thermal phenomena[17].

DSC measurements can be power compensation DSC or heat flux DSC. By using individual heating ele-

ments, both the sample and reference materials are kept at same temperature, difference being only the power inputs. This method is termed as power compensation DSC. To some extent, heat-flux DSC resembles differential thermal analysis (DTA)[18] where only heat differences between sample and reference materials are measured.

Calibration of DSC instrument is carried out with standards supplied by NTIS (National Technical Information Service)[19]. Transition temperatures and heat of fusion are accurately known for these standards. Subsequently, the melting point and heat of fusion of any given compound or polymorph can be experimentally determined.

METHODOLOGY USED IN TGA

Thermogravimetric analysis involves the measure of thermally induced weight loss of a material as function of externally applied temperature[20]. This technique is most commonly used to study desolvation processes and compound decomposition, differentiating anhydrous forms from solvates. This technique can be used along with Karl Fischer Titration method[21] to determine total volatile content of a pharmaceutical substance besides the moisture content. Thermogravimetric thermogram would not record any change when thermal events are not associated with any mass change, for example, solid-solid transition, solid-liquid transitions etc. Greater stability can be attributed to compounds having higher decomposition temperature.

Thermogravimetric analysis involves continual recording of mass of a compound with an in-built microbalance as it is heated in a programmable furnace whose inside surfaces are resistant to the gases evolved during analysis. TGA also depends on instrumental and sample related factors. For example, sample size, particle size, sample packing, thermal conductivity, the heats of reaction and nature of evolved gases affect the quality of the data. Thermal reactions also depend on the atmosphere in the furnace and furnace heating rate can cause a change in transition temperature. Performance of the Thermogravimetric Analyzer can be optimized by calibrating the reaction temperature across the reaction zone within the furnace for the required range of temperature. Reproducibility in TGA results is very important before any conclusion is made about a solvate.

Analysis of the evolved gases emerging in the gas flow from the reaction zone of TGA furnace can be accomplished by transporting them with low molecular weight gas having high thermal conductivity from furnace exit to other sensitive techniques like gas chromatography (GC) with flame ionization detector or katharometer measuring thermal conductivity. By interposing suitable cold traps between furnace and detector, the evolved gases like carbon dioxide, sulphur dioxide and even water can be condensed and later subsequent volatilization of the condensed gases can be used to estimate their compositions. GC-MS can be used for more accurate analysis where the evolved volatile products can be quantified with mass spectrometer as the sensitive detector[22]. Infra-red spectroscopy can also be coupled to TGA analysis and can be helpful to measure the actual water content.

ROLE OF DSC IN THERMODYNAMIC AND KINETIC STABILITY

Thermodynamic stability of the particular polymorph over the other and to acquire this stable solid-state, are important issues while studying polymorphism. These rules differentiate enantiotropic and monotropic polymorphs. If one polymorph is stable over a certain temperature range and pressure while another polymorph is stable over a different temperature range and pressure, polymorphs are said to be enantiotropic (Kuhnert-Brandstatter)[23]. Below the melting point of either polymorph enantiotropic polymorphs can be interconverted, since free energy curves cross before the melting point of either polymorph is reached. Example of this system includes carbamazepine, metochlopramide and tolbutamide. It is quite often that only one polymorph is stable at all temperature below the melting point and all other polymorphs are unstable. Such polymorphs are termed as monotropic. Free energy curves of monotropic system do not cross and therefore reversible interconversion is usually not observed below the melting point. The polymorphs which have higher free energy curve and solubility at a given temperature and pressure, are always unstable. For example, metolazone and chloramphenicol.

Burger and Ramberger thermodynamic rules are

decisive in differentiating enantiotropes and monotropes. Important of these rules are heat of transition, heat of fusion and density rule. DSC plays a vital role in using the first two rules for distinguishing such systems. If an endothermic transition between crystal forms is observed at a particular temperature, there exists a transition point below this temperature. Such polymorphs are interrelated as enantiotropes. If the transition observed is exothermic in nature at a particular temperature and no transition occurs at a higher temperature, polymorphs are said to be related as monotrops. Heat of fusion rule is important when DSC is not able to measure the heat of transition and the rate of transformation is slow. If the higher melting form has the lower heat of fusion, the polymorphs are enantiotropically related and if higher heat of fusion is exhibited by the higher melting polymorphs the forms are classified as monotrops. Polymorphic forms, form I and form II of Nabumetone[24] are good examples of monotropic system. Form I has a higher heat of fusion (31.3 kJ/mole) and melts at 15°C higher than form II which has lower heat of fusion (24.5 KJ/mole). This rule may not be valid in case polymorphs where their melting points differ by more than 30°C.

The Density rule[25] is based on the close packing of atoms of different molecules resulting in lowering of energy hence polymorph which lacks directional intermolecular interaction at 0° K will have the higher density. This rule is valid for those polymorphic forms where van der Waals interactions are more prevalent. For example density of Nabumetone form I (1.26 g/cm^3) is higher than form II (1.21 g/cm^3). Exception to this rule will be observed in those crystals where directionality is introduced by hydrogen bonding, enhancing the stability of the resulting polymorph. Retonavir polymorphic form II, although more stable, has a lower density than form I[26].

Ostwald law of stages[27] is important in deciding the order of appearance of polymorphs in crystallization process. Evaluation of solid phases is governed by kinetics of nucleation process and typical experimental conditions. Crystallization involves both nucleation and growth of a phase. Studies of growth kinetics and crystal morphology are important in identifying additives or solvent that enhances the crystallization of a particular polymorphic form[28]. The efficacy of crystal seeding in controlling the formation of a suitable polymorph depends on potential of the crystal surfaces[29]. Some times the insoluble degradation product can also promote the nucleation of more stable polymorph[30]. The approach of solvent selection for screening polymorphs, rate of nucleation order of appearance of polymorphs are all affected by kinetics, thermodynamics and diversity of molecular arrays formed by the active pharmaceutical ingredient molecules, for example carbamazapine[31] and sulphathiazole[32]. A fast nucleation rate is observed for those solvents which have high solubility but moderate solvent interaction. Thermodynamic factors and strength of solvent solute interaction are important when studying selective polymorphic nucleation. In some of the cases a disordered state for example amorphous drug substances are considered to be important precursor to a suitable crystalline state[33].

PHARMACEUTICAL APPLICATIONS OF DSC AND TGA

Identification of different polymorphs, establishing the stability of a particular polymorph, and optimizing processing conditions for preparing polymorph are crucial and important issues from patent as well as regulatory point of view[34]. Determining whether the polymorphic forms are monotropic or enantiotropic can be of immense importance in the formulation studies. Using Burger-Ramberger rule[35] one can ascertain the stability of a particular polymorph at any stated temperature. Applications and importance of these rules for the active pharmaceutical ingredients and drug excipients are described here in detail.

Chloramphenical palmitate is an antibiotic and known to exhibit polymorphism and exists in three forms, A, B and C. All the forms[36] were investigated with X-ray powder diffraction and DSC. Form A is stable at room temperature. Form B is metastable and Form C is unstable. The DSC thermograms of these three forms indicate endothermic peaks corresponding to melting point at 90.3°C for form A, at 86.7°C for Form B, and an exothermic peak for form C at 64.5°C followed by endothermic melting point peak at 86.3°C respectively. Endothermic peak after 86°C indicates that forms B and C are monotropic. The conversion of Form B to Form A was found to be exothermic in nature. Transformation indicated that forms B and A of chloramphenicol palmitate are monotropic. The presence of poly-

morphic form A in form B was also investigated while studying polymorphic mixtures of chloramphenical palmitate. On storage at 82°C, form B was converted into Form A, indicating that form B is metastable at this temperature. On grinding form B and form C were converted into form A which was ascertained by both XRD and DSC studies. Hence, using XRD as a complementary technique, DSC can provide detailed profile of the polymorphs of any pharmaceutical drug substance.

Grinding at different temperature can have a great impact on solid-solid transition behavior. Grinding of indomethacin[37] at 4°C resulted in an amorphous form but grinding at 30°C, a metastable form was formed. Grinding of cephalexin resulted in the formation of amorphous form.

Two polymorphic forms A and B of metoclopramide[38] were found to be enantiotropic. In DSC thermogram two endothermic peaks were observed for form A. At 125°C endothermic peak corresponded to melting of solid-solid transition to form B followed by endothermic peak at 147°C indicating melting of form B. Although the cooling resulted in stable Form B, on storage for three days at room temperature resulted in conversion to stable Form A indicating more stability of this form at room temperature. Monotropy in the three polymorphic forms of dehydroepiandrosterone polymorphs was ascertained using Burger's rule. In this case higher melting form was found to have higher heat of fusion.

Piretanide when recrystallized with twenty seven solvents, it formed six solvate and two anhydrous forms. Unique X-ray powder diffraction patterns of these solvates and anhydrous forms confirmed their crystalline behavior. When DSC was used to study their desolvation profiles, they ultimately led to the formation of one of the anhydrous form. One of the anhydrous form was also found to convert to another stable anhydrous form when subjected to DSC analysis.

Drug-excipient interaction has also been extensively studied with DSC analysis[39]. In this study the binary mixtures of each ingredient or API and an excipient are mixed and any alteration in melting endotherms is monitored. The information obtained from such studies indicates the chemical or physical interaction between API and excipient or two excipients. DSC analysis was performed for famotidine[40] and excipients. It was found that drug was compatible with talc, magnesium stearate, and avicel PH 101 but drug-excipient incompatible interactions were observed for kollidone, encompress, crospovidon and lactose. Formation of amorphous solid solution in later caused suppression of thermal features of famotidine.

Heating rate is crucial and decisive parameter in DSC study. Different DSC thermograms are obtained at different heating rates. Therefore, it is always advisable to carry out DSC analysis at different heating rates[41].

TGA can be used to prove the anhydrous nature of a polymorph. The characterization of solvates and hydrates is the prime concern of thermogravimetric analyses[42]. TGA is more convenient to perform than Karl Fischer analysis. It is a simple, conceptually applicable rapid method for weight loss determination. Weight loss in the region 80 to 100°C is commonly ascribed to water loss. The weight loss in the same region can also be attributed to volatilization or residual solvent or polymorph decomposition. TGA when run with DSC can be potentially highly useful analytical technique[43]. The loss of water of pharmaceutical hydrates occurs usually within 80° to 140°C. For most low molecular weight hydrophobic drugs, loss of sorbed water can be either in the range 20° to 30°C or 60° to 90°C, corresponding to an amount of less than 2% w/w. The amount can be higher for hydrophilic and high molecular substances.

In principle hydrates are crystalline materials in which water molecule is incorporated into the crystal lattice. Pharmaceutical hydrates can classified into three categories[44], isolated site hydrates, channel hydrates and ion-associated hydrates. In isolated site hydrates, drug molecules intervene and isolate the water molecules from each other. TGA profile for such system is narrow over a short temperature range and results in sudden stoichiometric water loss. In channel hydrates, the water molecule is present in channels made by drug molecules. Water loss for such system starts at a lower temperature than isolated site hydrates system and occur over a wide temperature range. Third class, ion-associated hydrates contain metal ions and are of considerable significance since a good salt selection process leads to good dissolution profile. Since a strong bonding exists between ion and water molecule, ion-associated hydrates lose water at very high temperature. TGA has been used as a routine method for char-

acterizing such hydrates.

Cephradine dihydrate[45] belongs to isolated site hydrates. Single crystal data indicates that each unit cell contains two molecules of cephradine and four molecules of water. Each pair of water molecules are isolated and regularly arranged in the in the lattice and form hydrogen bond with each other and also with carbonyl, carboxyl, and amide groups present on two cephradine molecules. Dehydration of the hydrate yields amorphous form which is highly unstable. Two unsresolved sharp endotherms are observed at 92.09° and 99.51°C and at the same temperature range a sharp weight loss was observed. Sharp O-H stretches at around 3520 and 3425 cm^{-1} are observed in the diffuse reflectance infrared spectrum of cephradine dihydrate but not im amhydrous form. The information obtained with DSC, TGA and DRIFT analytical methods and single crystal studies indicate that no network of hydrogen bond exists on any axis through the crystal lattice. Examples of channel hydrates include ampicillin trihydrate[46].

Sesquihydrate of on of the enantiomer of hyoscine hydrobromide[47], an anticholinergic drug isolated from the belladone plant, was found to be conformationally similar to hemihydrate in unit cell dimension, packing arrangement and crystal system when studied with XRD. The drug exhibits polymorphism and both sesquihydrate and anhydrous forms exist. TGA analysis on the other hand confirmed the total mass loss of 6% w/w indicating 1.5 molecule of water per molecule of the drug substance. Loss of one molecule was observed at about 90°C and half a molecule of water at 102°C. Hence, TGA can not only be used as complementary technique but also provide a great insight into the hydrate crystals.

Complementary arrays of analytical techniques, including TGA, DSC, Karl Fischer Titrimetry, X-ray powder diffraction, scanning electrode microscopy (SEM), FT-IR, and ^{13}C SS-NMR were used to investigate nedocromil magnesium hydrates[48], the drug used in the treatment of asthma. Several hydrates of this drug are known and three of them that is heptahydrate, decahydrate and pentahydrate were subjected to TGA analysis. For heptahydrate and decahydrate, the water loss started immediately after heating from ambient conditions and biphasic mass loss was observed in their derivative curve. Spectroscopic studies hint similar structure for both heptahydrate and decahydrate indicating three molecule of water are loosle bound in decahydrate. Both these forms were found to be less crystalline than pentahydrate. For pentahydrate, the loss was observed in two stages, that is loss of four water molecules at 75-130°C and one molecule at 200-230°C. From spec troscopic studies it was confirmed that that with four water molecules, pentahydrate exists as tertramer and with one molecule of water as monomer. With TGA it was further confirmed that tertramer was comparatively less stable than the monomer. The unique XRD pattern with characteristic diffraction peaks for pentahydrate differentiates it with other hydrates. Hence, in combination with spectroscopic techniques TGA provide specific structural information about complex pharmaceutical hydrates.

Dialkylhydroxypyridones[49], iron chelators, with possible use in the treatment of anemia, form solvate with formic acid. Desolvation profiles of these substances when studied with TGA confirmed the loss of formic acid and molecular information were subsequently confirmed with spectroscopic techniques.

TGA has also been used to study the excipient forming hydrates and drug-excipient interaction. α,α-trehalose (α-D-glucopyranosyl α-D-glucopyranoside) is a nonreducing disaccharide. To elucidate the mechanism by which trehalose enable biological organisms to survive dehydration stress, it becomes necessary to study various solid-state modifications and also their transition from one form to other. Three forms of trehalose have been recognized which include Form I (T_h or dihydrate), II (Tα or anhydrous form) and III (Tβ, other anhydrous form). Amorphous state has also been observed. One more form has also been identified[50] as Tγ. It was observed that trehalose dihydrate, with smaller particle size, on dehydration leads to amorphous material whereas larger particle formed anhydrous, Tβ form. Dehydration profile of dihydrate form depends on atmospheric pressure, purge gas flow, and the kind of pans used to study DSC and TGA.

When DSC analysis was performed with pin holed pans[51] baseline discontinuity was observed between two endothermic peaks. The concomitant TGA analysis performed on dihydrate form revealed water loss was associated with second endothermic peak. The isolation of material formed after first endothermic peak was confirmed as Tγ form. Tγ form was hence considered to be

the mixture of dihydrate and anhydrous forms or a partially dehydrated system in which anhydrous layer prevented further water loss. On further heating the water loss was observed in second endothermic peak confirmed by TGA studies. In drug-excipient studies it was observed that a simple analgesic drug like paracetamol lowered the termperature needed to recrystallize amorphous state of trehalose to anhydrous form[52].

ROLE OF DSC IN STUDY OF AMORPHOUS MATERIALS

Amorphous solids exhibit short range order but lack three dimension long range molecular order possessed by crystalline polymorph[53]. These amorphous solid modification have high free energy and faster dissolution rate. A term polyamophism has been attributed to certain amorphous materials where existence of two glassy forms of same compound differ in frisalty has been reported. Usally a strong glass former has a low density and fresile glass former posses high density. These compounds can be characterised by first order transition between to amorphous state for example freeze drying and spray drying of Cefamandole Nafate[54] leads to amorpous states having differences in X-ray powder differaction patterns.

There is a considerable interest in determination of glass transition temperature, T_g in DSC analysis of drug substance as well as excipients[55]. Plasticizer such as water has a great effect on glass transition temperature T_g. The DSC studies have been performed on such systems to find out T_g which indicate a change in relaxation behavior of the drug substance or excipient. T_g can be regarded as a measure of physical and chemical stability of the amorphous material. Increased molecular mobility of the amorphous drug substance or excipient causes recrystallization.

Majority of DSC studies on spray dried systems[56] suggests the formation of amorphous material. Example includes, thiazide diuretics[57], digitoxin[58] etc. Freeze-dried methods[59] are generally used for the preparation of injectables in a dry form which can be easily reconstituted. The process involves initial freezing followed by primary and secondary drying ultimately forming a glassy system with a high viscosity. This glassy system is of major importance in pharmaceutical industry and the process is usually used for heat-sensitive drugs. Glass transition of such glassy systems has been extensively investigated with DSC.

Amorphous of tri-O-methyl-cyclodextrin[60] prepared by melt rapid quench and milling process have different crystallization rates despite having the similar glass transition and Heat Capacity temperature. Instability of amorphous state can lead to formation of different crystalline forms for example lower than than glass transition produce the gama polymorph where as above this temperature alpha form in mainly produced. Long term stability of amorphous state is evaluated by those technique which reflect in molecular interaction and molecular mobility like enthalpy relaxation.

HOT STAGE MICROSCOPY

Hot stage microscopy or thermal microscopy[61] is a rapid method for studying existence of polymorphism and screening polymorphs. This technique uses polarized light and during heating any change in any optical property signifies a phase change and hence melting point differences. The instrument used for this technique is hot stage of Kofler[62].

This technique requires only a few milligrams of API or an excipient. The substance is kept on a microscopic slide and subjected to heating and cooling processes. Complete phase diagram can then be generated based on all the observations made during thermal processes. This technique can be used to determine the melting point with great accuracy and to infer the nature of a polymorphic transition. An enantiotropic substance is distinguished by its ability to transform reversibly into another phase. An irreversible nature of this transition indicates monotropic nature of the substance.

PHARMACEUTICAL APPLICATION OF HOT STAGE MICROSCOPY

Prednisone acetate polymorphic form II melts over the temperature range 225-228°C on a hot stage[63]. On further heating the melt gets solidified and again melts over a temperature range of 232-241°C which is actually the melting point of prednisone acetate Form I.

Corticosterone-21-acetate Form II when heated to 140°C on a hot stage[64], it melts over a temperature

range 145-148°C. On further heating, the melt solidifies and is converted to Form I. Form I on further heating melts at 153-155°C.

Metformin hydrochloride Form B[65] is transformed to needle-shaped crystals of Form A when heated to 80°C. Generation of needle-like crystals of meta-stable Form I of Caffeine[66] on dispersion in Gelucire 50/13 from stable Form II, indicates enantiotropic polymorphic system.

From these clear visible changes and melting point determinations, it is obvious that hot-stage microscopy can be an extremely valuable analytical technique in characterization of polymorphic systems. FT-IR and Raman spectroscopic techniques can also be concomitantly used with thermal microscopy and valuable information can be obtained in studying polymorphism. Paracetamol and lufenuron are the examples of such study where Raman spectroscopy has been used besides the thermal studies[67].

MISCELLANEOUS THERMOANALYTICAL TECHNIQUES

There are certain other techniques which show considerable potential for polymorphic evaluation include are either modifications of above mentioned technique or based on temperature dependent thermodynamic parameter of the polymorph. For example, MTDSC, modulated temperature differential scanning calorimetry[68], is a software modification of conventional DSC. In this technique a perturbation, for example a sine wave, is superimposed on the conventional linear signal. The instrument gives three kinds of signals including heat capacity, kinetic and total heat components. This method is preferentially used for amorphous systems. MTDSC has been used for studying glassy behavior of nifedipine[69] and itraconazole[70]. This technique has also been used for studying enantiotropic transition of caffeine[71].

Other advanced DSC-based technique includes Hyper-DSC™, also called High-speed DSC technique[72]. This technique utilizes high scanning speeds which make it easier to identify the metastable forms. For example, endotherm, corresponding to carbamazepine Form III, a metastable form, could be isolated with such studies[73].

Microthermal analysis is an interface technique used to differentiated crystal polymorphs. In this technique the tip of an atomic force microscope is replaced by a small thermistor and analysis can be performed on polymorph with a size of few square microns[74]. This technique is also called highly localized DSC. For example, cimetidine[75], indomethacin[76] and caffeine[77] are some of the examples which have been investigated with this localized thermal analysis (LTA) technique[78].

Thermally stimulated current (TSC)[79] which has a widespread use in polymer industry is now extensively used in polymorphic characterization. This technique, at elevated temperature which is above the transition temperature, electric filed is applied to the sample followed by rapid cooling. This cooling "freezes" polarization in the sample. The sample when reheated a current due to movement of dipoles in the sample is recorded as a function of temperature. Movement of dipoles arises due to the thermal rearrangement observed during heating process. For example, irbesartan forms A and B gives different TSC profiles[80].

Heat of solution is one of the physical properties of the polymorphic system. When a solid is dissolved in a known volume of solvent, enthalpy of dissolution can be determined. Heat of solution[81] is extremely sensitive to kinds of crystal lattice, hence polymorphic studies can be performed for those polymorphs whose melting points are very close but differ in enthalpy of dissolution. One such example of this study is characterization of cimetidine polymorphs[82].

CONCLUSION

Since polymorphs have different physical properties it is often advantageous to choose the proper polymorph for the suitable pharmaceutical application. The fundamental questions associated with polymorphism in pharmaceutical sector are solubility of each form, purity achieved in producing the stable crystals, and extent to which the required form remains stable during the processing stages like micronization, tableting, lyophilisation etc. One of the important consideration on which a great emphasis is laid is the number of polymorphs a given API can have. Physical and chemical stability of each polymorph is also a matter of grave concern. Certain polymorphs are known to exist in metastable states which are equally important in pharmaceutical in-

dustry but whether these states can be stabilized remains many a times an unanswered question. For identification of the solvates, DSC, TGA and hyphenated techniques are very important. Hot stage microscopy is a rapid method for studying existence of polymorphism and screening polymorphs. Alteration in DSC techniques itself has generated several discipline to investigate polymorphs and amorphous substances in great detail.

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