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Review

Methods of amorphization and investigation of the amorphous state

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The amorphous form of pharmaceutical materials represents the most energetic solid state of a material. It provides advantages in terms of dissolution rate and bioavailability. This review presents the methods of solid-state amorphization described in literature (supercooling of liquids, milling, lyophilization, spray drying, dehydration of crystalline hydrates), with the emphasis on milling. Furthermore, we describe how amorphous state of pharmaceuticals differ depending on the method of preparation and how these differences can be screened by a variety of spectroscopic (X-ray powder diffraction, solid state nuclear magnetic resonance, atomic pairwise distribution, infrared spectroscopy, terahertz spectroscopy) and calorimetry methods.

Keywords: amorphous solid preparation, crystallinity, spectroscopy, calorimetry

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The existence of drugs and excipients in multiple physical forms (*e.g.*, polymorphs) provides pharmaceutical scientists with an opportunity to select the preferred form of the material to be used in a formulation. This is very useful since critical properties, such as particle morphology and dissolution properties, are frequently different in the different physical forms of a material. The amorphous form of pharmacologically active materials has received considerable attention because, in theory, it represents the most energetic solid state of a material, and should thus provide the biggest advantages in terms of dissolution rate and bioavailability (1). However, the amorphous form also shows various disadvantages, such as lower physical stability compared to crystals.

The structure of an amorphous solid is usually described as possessing a crystal-like short-range molecular arrangement, but lacking a long-range order. As illustrated by Fig. 1, the immediate environment of a molecule (m) in an amorphous solid may not differ significantly from that in a crystal (*e.g.*, similar number of and distance to nearest neighbors), but an amorphous solid lacks any long-range transational-orientational symmetry that characterizes a crystal (1). However, this is only true when we are dealing

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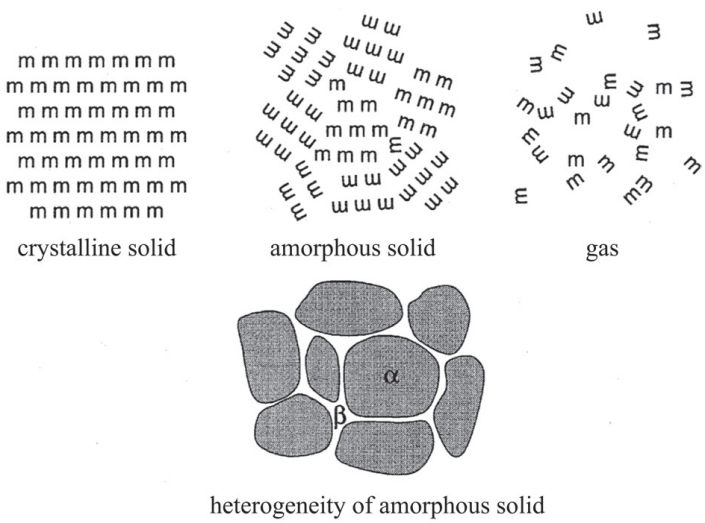

Fig. 1. Schematic representation of the structure of an amorphous and a crystalline solid. Molecular arrangement in the amorphous solid is not totally random, like in the gas phase, but features a short-range molecular order similar to that in the crystalline one. According to some models, an amorphous solid has distinct regions (*e.g.*, α and β), which have different densities and thermal relaxation behaviors (*i.e.*, the time scale for long-range molecular motion). Adapted from ref. 2 with permission.

with non-ionic material and substances that do not form strong intermolecular connections with water.

In Fig. 2, the enthalpy (H) or specific volume (V) of a solid substance is plotted as a function of temperature. For crystalline material, at very low temperatures we see a small increase in enthalpy and volume with respect to temperature, which is indicative of a certain heat capacity (C_p) and thermal expansion coefficient. There is a discontinuity in both H and V at the melting temperature T_m representing the first order phase transition to the liquid state. Upon rapid cooling of the melt, the values of H and V may follow

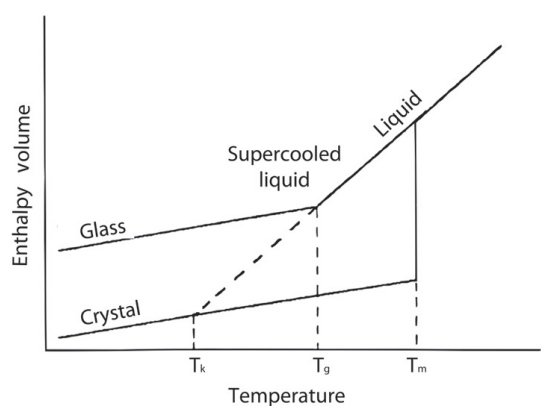

Fig. 2. Schematic description of the variation in enthalpy (or volume) with temperature T_m, T_g, T_k. Adapted from ref. 1 with permission.

the equilibrium line for the liquid beyond the melting temperature into a »super-cooled liquid« region. On further cooling, a change in slope is usually seen at the characteristic temperature known as the glass transition temperature (T_g). At T_g, the properties of the glassy material deviate from those of the equilibrium super-cooled liquid to give a non-equilibrium state having even higher H and V than the super-cooled liquid. The Kauzmann temperature (T_k) is thought to mark the lower limit of the experimental glass transition (T_g) (1).

Amorphous materials have higher Gibbs free energy than their crystalline counterparts, and as a result have higher apparent solubility and faster dissolution rates, which in turn can lead to higher bioavailability for drugs that exhibit dissolution-rate limited absorption (classified according to the Biopharmaceutical Classification System (BSC) as class II drugs) (3). However, due to excess entropy, enthalpy and free energy that account for better solubility, the amorphous state is inherently unstable and recrystallization may occur (2).

METHODS OF AMORPHIZATION

The most common techniques of preparing amorphous forms in pharmaceutical systems (*e.g.*, pure drugs or polymer glass solutions) can be categorized according to two principal transformation mechanisms. In most cases, crystalline material is intermediately transformed into a thermodynamically unstable non-crystalline form (either a melt or a solution). Further, the thermodynamically unstable amorphous solid material is prepared by quench cooling of the melt or rapid precipitation from solution, *e.g.*, spray drying. The other transformation mechanism involves direct solid conversion from the crystalline to the amorphous form. The best example is mechanical activation such as milling. Whilst when melt and solution mediated methods are used all crystallinity is lost in the intermediate phase (melt, solution), mechanical activation methods may not cause complete disruption of the molecular order (1, 4–6).

It should also be noted that differently prepared amorphous form exhibits different properties. Salvonien *et al.* (7) have found that the cryo-milled simvastatin has lower stability and a reduced recrystallization rate than quench cooled samples of the same substance. In comparison, Karmwar *et al.* (8) found that the cryo-milled amorphous form of indomethacin is the least stable compared to amorphous form prepared by quench cooling and spray drying. Moreover, it should be noted, the differences between the differently prepared amorphous forms are not only in the stability but can be detected on the molecular level using terahertz spectroscopy or solid state NMR (ss-NMR) (9, 10).

The term polyamorphism has been used to describe amorphous states produced by different annealing times or preparative routes. An example are glasses that have been aged below T_g for different times and hence developed various degrees of »relaxation enthalpy«, that is, the enthalpy of an aged amorphous substance (2). The term polyamorphism has also been used in literature to describe the existence of distinct amorphous phases separated by first-order phase transitions (4, 11). Patterson *et al.* (12) have shown that the susceptibility to amorphous conversion by different methods is compound specific. Therefore if amorphous conversion is required, there is merit in investigating the use of more than one preparative technique.

Besides the possibility of the existence of polyamorphs for pharmaceutically relevant substances, the concept of a rigid amorphous fraction (RAF) has also been introduced. RAF is believed to be an intermediate between the crystalline and amorphous phases. The striking difference between RAF and the »true« amorphous fraction lies in the change of heat capacity (ΔC_p) at the glass transition (T_g). As RAF is more closely associated with the crystalline state, it does not undergo a change in C_p and T_g. This implies that an amorphous form containing RAF will exhibit a smaller change in C_p and T_p than the same amorphous substance without RAF (13). Solid-state amorphization will be the main topic addressed in this paper.

Solution based methods of amorphization

Melting and quench cooling. – Melting and quench cooling of a crystalline drug to produce an amorphous product is a relatively simple technique. Upon cooling of the molten drug below the freezing point, molecular motion is slowed down. If cooling of the molten drug takes place fast enough, the molecules are not able to rearrange into a crystalline lattice; therefore, they are »frozen« in a more disordered state and crystallization is avoided (14, 15). The slower a liquid is cooled, the longer is the time available for configurational sampling at each temperature, and the colder it can become before falling out of liquid (7). Therefore the enthalpy of the end product depends on the cooling rate (16) although it must be noted that if the cooling rate is too slow, the product will crystallize. The drawback of this approach is its potential for chemical degradation during the melting step due to high temperatures, since the degradation products may lower the glass transition temperature of the amorphous product (17). Thermal degradation is compound dependent and only limited steps can be taken to overcome this problem, such as heating under an inert gas (12). So far, a range of pharmaceutical active substances and excipients have been successfully amorphized by melting and quenching (18–21).

Spray drying. – The spray drying technique may be suitable for obtaining the amorphous form of the drug substance, either alone or in combination with the polymer (22–25). It converts a liquid solution into a powder in one step (26). Concentrated liquid is pumped to the atomizing device where it is broken into small droplets. These droplets meet a stream of hot air and lose the solvent very rapidly while still suspended in dry air. Components of the concentrated liquid may not crystallize immediately when their solubility limit is reached at the droplet surface. In this case, a fully or partially amorphous solid is formed (27). Drugs that have a relatively low T_g make it very difficult to obtain a stable amorphous product in the form of free flowing powder by spray drying. As the outlet temperature rises above the T_g, there is always a possibility that the final product is present in the super cooled rubbery state. Also, such product is often sticky or tacky, which causes a decrease in product recovery and hampers its handling in subsequent processes (22). Spray drying has been commonly used in the past decade as a technique for preparing amorphous active substances and excipients (28–31).

Freeze-drying. – Freeze-drying, also known as lyophilization, has been used for a number of years as a pharmaceutical unit operation for the low temperature drying of injectable systems (32). Freeze-drying involves desiccation of a substance by crystalliza-

tion of water, followed by sublimation of water vapor from the solid state at reduced pressure. Depending on the cooling rate, some solutes may crystallize during the freezing stage. Those solutes which do not crystallize are converted to amorphous solids when the temperature drops below the T_g of the maximally concentrated solute. At the end of a freeze-drying process, when the solvent is completely removed through sublimation, the freeze-dried formulation exists as an amorphous or partially amorphous system. The T_g of a freeze-dried formulation is determined by components of the formulation and the presence of residual water, which can act as a plasticizer lowering the glass transition temperature (33). The process has been recently utilized to produce amorphous trehalose and itraconazole (34, 35).

Solid state amorphization

Dehydration of crystalline hydrates. – Dehydration of crystalline hydrates has been demonstrated as a feasible and »gentle« route to the amorphous state of organic solids. Saleki-Gerhardt *et al.* (5) showed that heating crystalline raffinose pentahydrate at 60 °C in vacuum converts the material to an amorphous form identical to the one produced by freeze-drying (5). Li *et al.* (36) observed that crystalline carbamazepine dihydrate becomes amorphous upon dehydration at 45 °C with N_2 purge. The resulting amorphous solid undergoes a glass transition at 56 °C, which is markedly above the drying temperature (45 °C), and crystallizes on further heating (at 86 °C). These studies indicate that apart from being a potential route to amorphous solids, the drying of crystalline hydrates may reduce their physicochemical stability through loss of crystallinity. More recently, Sussich *et al.* (37) investigated the amorphization of a di-hydrate crystalline polymorph of trehalose upon dehydration.

Milling. – Milling, also known as comminution or grinding, is typically regarded as a particle size reduction process across many industries, including the manufacturing of pharmaceuticals and fine chemicals (38, 39). In secondary pharmaceutical processing, it is often used to increase the specific surface area of poorly water-soluble drugs to improve their dissolution properties and bioavailability (40). There is a variety of different commercially available milling equipment that can be utilized for the communition of pharmaceuticals. In general, the devices can be subdivided into three main categories, based on how energy is transferred to the material to be ground: ball mills, shear action mills and shock action mills. In the case of ball mills, energy is transferred to the ground material by mill bodies or impellers. The material is exposed to both shear and normal stresses. On the other hand, in shear action mills, the material is ground by crushing elements (solid surfaces in relative motion). Shock action mills transfer the energy directly to the milled material. In this case, material is milled by direct collision of particles. However, besides reducing the particle size, the milling process is often accompanied by other unintended effects such as changes in morphology (41), crystallinity (41), polymorphism (42), glass transition temperatures (43), chemical stability (44) and melting behavior (45) during subsequent post-milling storage. The process of milling is very energy-consuming. Furthermore, the high energy output could result in degradation of the milled substance and the milling equipment. Milling is also used for more specific applications such as preparation of co-crystals (46, 47). Pharmaceutical literature describes numerous examples of organic compounds like piroxicam (48), budesonide (49), sucrose

(50, 51), lactose (52, 53), trehalose (54, 55), that become partially or completely amorphous when submitted to milling. However, it appears that one of the disadvantages of amorphization through milling is that the T_g of the milled substance plays a fundamental role and sufficiently low temperatures of milling (T_{mill}) must therefore be provided to induce amorphization through milling (56).

Principles of solid-state amorphization through milling. – There are several competing ideas as to how the transformation from the crystalline to the amorphous state takes place during milling. A commonly held view is that amorphization occurs *via* generation of localized heating effects followed by quenching. Alternatively, Okamoto *et al.* (57) suggest that milling leads to an increase in static disorder that adds to the intrinsic dynamic disorder inherent within the lattice up to critical value where the structural collapse occurs. Others argue that the disordering process is best regarded as an accumulation of defects (or, viewed another way, a dramatic reduction in crystallite size) and hence may not necessarily be regarded as amorphization in the traditional sense (58). Some of the advantages of cryogenic milling are that it is a mild method of producing amorphous material without the use of solvents or melting.

Influence of temperature on solid-state amorphization through milling. – The group of Descamps *et al.* (52, 59, 60) provides a very significant contribution on how the previously mentioned theories apply to organic crystalline materials. They have highlighted the relationship between the temperature of milling and the glass transition temperature of the corresponding material. In particular, they have demonstrated that cryo-milling (well below T_g) results in amorphization and milling above T_g results in polymorphic transformation. This contradicts the melt-quenching explanation. These authors favor the concept of driven materials, as outlined by Martin and Bellon (61). In brief, the approach suggests that in milling a material there is a temperature independent disordering process induced by ballistic interactions, which competes with the temperature dependent restoration process. The balance of these two processess is expressed in terms of an »effective« temperature T_{eff}, which is the temperature at which the non-milled material would assume the same level of disorder as the milled one. A milling process that resulted in a T_{eff} above the melting point would therefore be expected to result in amorphization. The value of T_{eff} may be estimated by *Eq.* (*1*) where T is the temperature of milling, D_{bal} is the (temperature independent) ballistic diffusion coefficient and D_{th} is the thermal diffusion coefficient corresponding to the restorative process.

$$T_{eff} = T(1+D_{ball}/D_{th}) \qquad (1)$$

It is significant that this theory predicts that at low temperatures D_{th} becomes small compared to D_{bal}, hence raising T_{eff}. This then explains the counterintuitive observation that amorphization to a greater extent occurs at lower temperatures (62).

A related question is whether the material generated by cryo-milling behaves in a similar manner to »conventionally« generated amorphous materials. Surprisingly, little is known about the subject, although a thorough study by Crowley and Zografi indicated that cryogenic milling of crystalline indomethacin resulted in amorphous material with similar T_g values but reduced physical stability compared to conventionally generated amorphous indomethacin. The authors ascribed the latter to the presence of residual

crystalline material (63). Qi *et al.* (62) have also demonstrated a very marked instability of the cryo-milled material below its T_g and also the possibility of the prepared amorphous materials exhibiting a more complex recrystallization profile than commonly considered for the solid state amorphous form.

The effect of crystal morphology on induced crystal disorder through milling. – One persistent challenge in the development of pharmaceutics is the crystal habit, or morphology. For example, equidimensional crystals are usually preferred in the industry as they have better handling and processing characteristics such as flowability and compactability (64, 65). Chikalia *et al.* (66) have found that β-succinic acid in a plate-like morphology is more prone to disorder than a needle-like morphology. Crystal morphology engineering and the use of crystals with the most suitable morphology could therefore be a valuable tool to enhance the solid-state transformation.

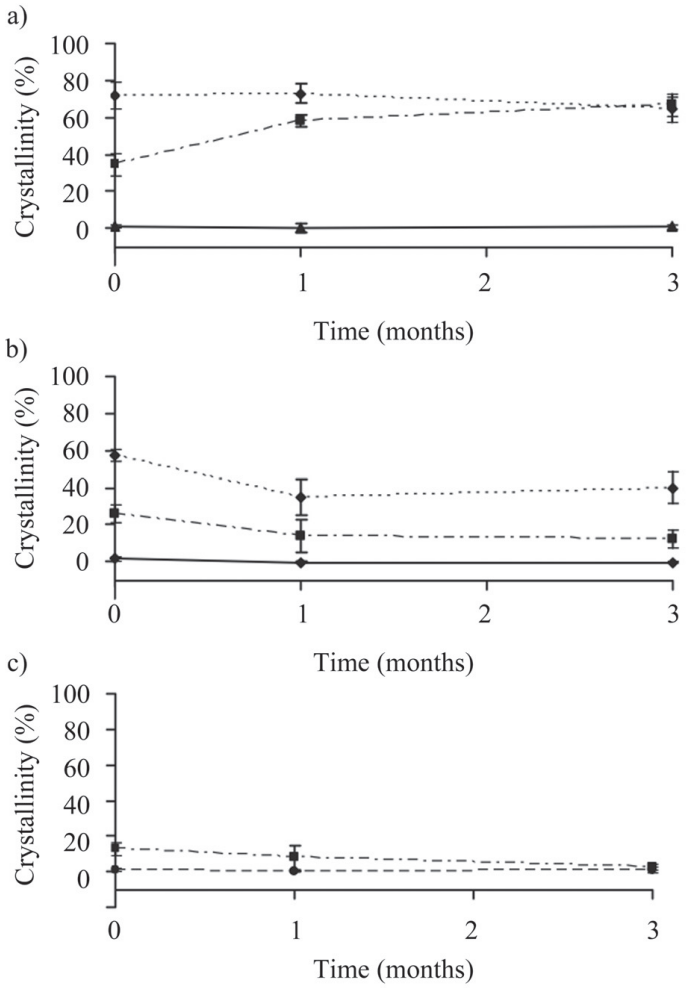

Fig. 3. Indomethacin co-ground with Neusilin in different ratios at 75 % RH and stored at 40 °C/75 % RH: a) 1:1, b) 1:4, c) 1:5. Key: 12 h co-ground, – · – · – 3 days co-ground, – – – 5 days co-ground, —— 8 days co-ground. Adapted from ref. 78 with permission.

Co-milling. – Co-milling of drugs with excipients has been employed for acceleration of solid amorphization and stabilization of amorphous state. Watanabe *et al.* (67) showed that amorphization of indomethacin could be achieved by milling it with polyvinylpyrrolidone or silica. Ali *et al.* (68) used a vibration mill to prepare amorphous co-ground mixtures of flufenamic acid with amorphous calcium silicate and silicon-dioxide. Amorphization of ibuprofen, sulfathiazole, phenothiazine, acridine, chloranil and vitamin K3 has been achieved by co-grinding with polyvinylpyrrolidone (69–71). Amobarbital amorphized in the presence of a variety of excipients such as carbon black, ethyl cellulose, precipitated silica and activated charcoal (72). A variety of other excipients such as β-cyclodextrin, dextrans, chitin, chitosan, gelatin, polyethylene glycol, methyl cellulose, hydroxyl propyl cellulose, calcium silicate and silicon dioxide were used to amorpize structurally diverse drugs, resulting in various degrees of amorphization (67–77). More recently, Bahl *et al.* (78) have shown that increasing the amount of Neusilin US2 with respect to indomethacin reduced the amorphization time (Fig. 3). The mixture also showed high physical stability.

Time of milling and solid state amorphization. – The crystal to glass conversion upon milling often requires milling times of several hours to complete (50, 54). Short milling times are thus expected to induce a size reduction of both the crystallites (small single crystals) and the particles (a particle can be composed of several crystallites) without generating any noticeable amorphous content. Until now, only a few investigations of weakly milled materials have been performed and little is known about the structural and micro-structural states that precede amorphization observed during long and intense milling (79). A very important point is to determine when and how the accumulation of crystalline defects (crystal surfaces, dislocations, vacancies, *etc.*) upon milling gives rise to a genuine amorphous state, which is, in turn, characterized by chemical disorder. Caron *et al.* (10, 80, 81) have studied the structural and thermodynamic changes of crystalline alpha-lactose in the course of its solid-state amorphization by milling. The results revealed that, in the course of the milling process, the material cannot be described as a biphasic system made of both perfect crystalline matter and genuine amorphous matter. It appears to be constituted of a wide panel of structural states more or less disordered and ranging from the crystalline state to the amorphous state. This conceptual difficulty clearly emerges from the results of these authors, who have shown that very different characterization techniques give rise to very different kinetics of amorphization (Table I). Terada *et al.* (82) have shown that peak intensities of the X-ray powder diffraction (XRPD) patterns of two polymorphs of terfenadine decreased with increasing grinding times (Fig. 4).

Table I. Differences in the time of amorphization depending on the method of solid-state characterization

	Indomethacin	Lactose	Trehalose
DSC	120 min	50 h	10 h
XRPD	30 min	1 h	No data
ss-NMR	195 min	30 h	20 h

Adapted from refs. 10, 80, 81 with permission.

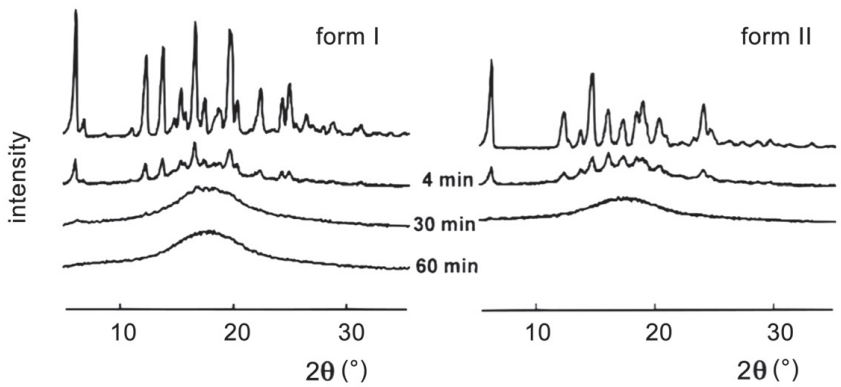

Fig. 4. Change in XRPD patterns of terfenadine (form I and form II) by grinding. Adapted from ref. 82 with permission.

CHARACTERIZATION OF THE AMORPHOUS STATE

The strategy of characterizing amorphous solids differs from that for crystalline solids. Molecular-level structural elucidation, which is feasible for crystalline solids by diffraction and spectroscopic methods, is less applicable to amorphous solids, and greater emphasis is placed on structural mobility and changes. It is customary to characterize amorphous material both below and above the glass temperature, *i.e.*, both as a frozen solid and a super-cooled viscous liquid. Thus effective characterization of amorphous pharmaceutical products requires a multidisciplinary approach using complementary analytical methods. To characterize pharmaceutical solids, we may use various techniques, such as Raman spectroscopy (RS), Fourier transform infrared spectroscopy (FT-IR) or solid state nuclear magnetic resonance (ss-NMR), which are primarily intramolecular methods probing the sample at the molecular level. Intermolecular information is gained by directly employing techniques such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and XRPD, which analyze the sample on the particulate level. Recently, tetrahertz pulsed spectroscopy (TPS), second harmonic generation (SHG) and ^{14}N nuclear quadrupole resonance (NQR) have been also used as spectroscopy techniques to directly investigate particulate properties of solids (81, 83–85). Other properties associated with the particulate level, such as morphology or size distribution, can be characterized using microscopic techniques such as polarizing light microscopy (PLM) and scanning electron microscopy (SEM) (84, 86). These methods offer several types of information about the investigated substance (87, 88):

(i) Structure: The structure of amorphous solids is not random at the molecular level, but may possess short-range order, residual crystallinity, polymorphic states, and regions of different density.

(ii) Thermodynamics: As mentioned before, amorphous solids present higher energy, entropy and free energy when compared to the crystalline state of the same material. Excess properties are parameters included in some theoretical models of crystallization and structural relaxation.

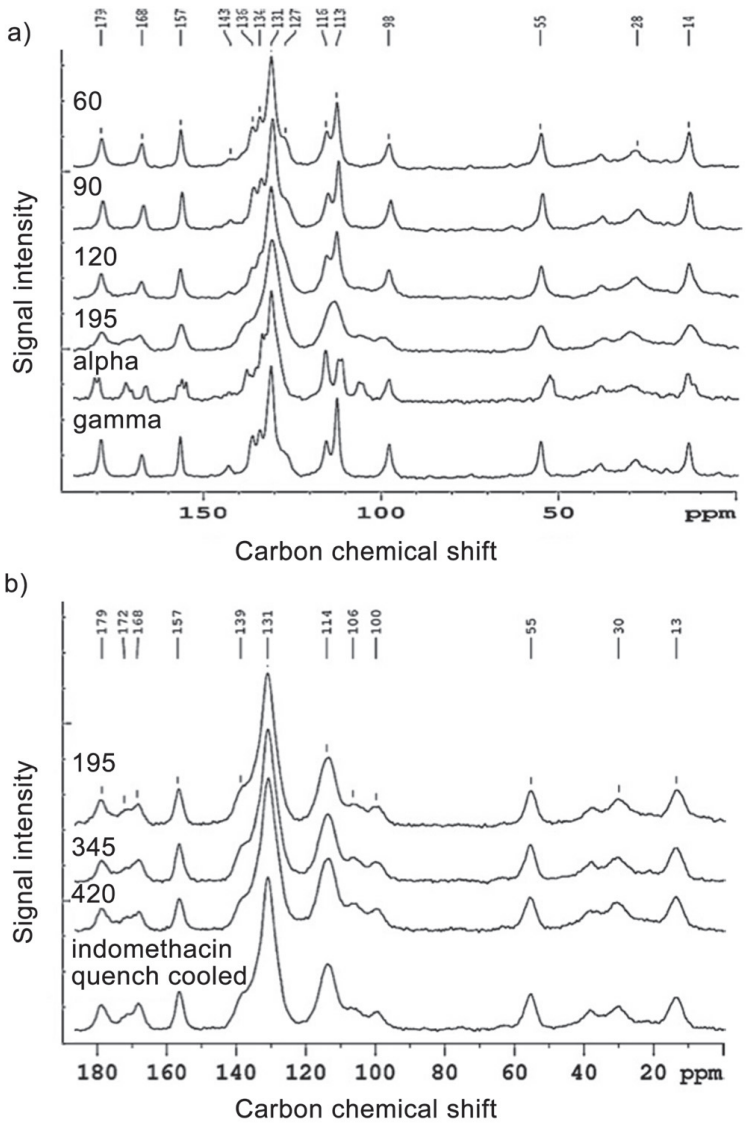

Fig. 5. NMR spectra of a) 60-, 90-, 120-, 195-min cryo-milled samples of γ-indomethacin and α-indomethacin; b) 195-, 345- and 420-min cryo-milled samples of γ-indomethacin and quench cooled amorphous indomethacin. The quench cooled sample and the cryo-milled samples with increasing time of milling show much broader peaks, which is characteristic of amorphous substances (81). Adapted from ref. 81 with permission.

(iii) Changes: When exposed to the right conditions amorphous solids can crystallize or undergo structural relaxation owning to instability compared to the corresponding crystals and »equilibrium« glasses.

(iv) Multi-component systems: Many pharmaceutical formulations are formed by either active substances and drug excipients or multiple active substances. One or more of the components can be present in the amorphous state.

ss-NMR

Recently, ss-NMR has been introduced to identify effects such as polymorphism, intra- and inter-molecular hydrogen bonding and tautomerism and is now widely used in conjunction with other analytical techniques (89). High-resolution ^{13}C ss-NMR spectra are obtained using proton decoupling and magic angle spinning (MAS) and sensitivity enhancement is achieved by cross-polarization (CP). ^{13}C ss-NMR has the advantage of being a nondestructive test method that provides information about the structure of the material (90). Furthermore, one of the advantages of ss-NMR is that it is very sensitive to minor conformational changes but is insensitive to particle size. In a ss-NMR study of ranitidine hydrochloride, the authors showed that form II of the drug exhibits molecular disorder crystals and contains two tautomers, nitronic acid and enamine (91). Molecular disorder was attributed to ranitidine hydrochloride solved intermolecular bonding (89). For the characterization of organic substances, the remarkable sensitivity of the ^{13}C chemical shift to local modulations of electronic density has made this technique one of the best probes of conformational aspects. In particular, ^{13}C CPMAS (cross polarization magic angle spinning) appears to be well adapted for studying poly- (or poorly) crystalline solids, revealing qualitative and quantitative features such as identification of phases and structural changes in crystalline polymorphs of pharmaceutical molecules (92–94). Recently, the development of proper analysis methods to measure relative amorphous and crystalline fractions has raised considerable interest (90, 95). Lefort *et al.* (10) have shown, in a study of ball milling trehalose, that an NMR approach can be readily implemented in many situations involving continuous transformations of pure compounds and can still remain a successful method for estimating amorphous content of a sample, even though DSC might fail at it (10). Furthermore, Bøtker *et al.* (81) utilized ss-NMR to explain the influence of different times of cryo-milling on the amorphization of indomethacin (Fig. 5).

X-ray powder diffraction

The principle behind XRPD experiments is the random orientation of crystals in a substance. If powdered crystals are randomly oriented, then for all sets of planes some of the crystals in the powder will be in the right orientation with respect to the X-ray source to satisfy Bragg's law for proper angle θ. What follows is that at least a few of the mineral grains in the powder will diffract for each of the planes during a scan through angles θ. The more finely ground the powder, the more likely it is that all orientations are sufficiently present. The whole XRPD method is based on the fact that ideally every possible crystalline orientation should be represented equally in a powdered sample. Two main types of powder diffraction experiments are possible according to Smith: automated powder diffractometer experiments yielding (digital) computer output and Debye-Sherer experiments providing (analog) film output. The Debye-Sherer approach uses a camera. A strip of film is wrapped around the powder sample so that diffracted beams from a fixed X-ray source can be recorded for all values of θ simultaneously. During the measurement, the powder diffractometer moves both the X-ray source and an electronic detector through arcs of θ values and sends to a computer periodic signals proportional to the averaged diffracted X-ray intensity. Both experiments provide the intensities for diffracted beams as a function of the diffraction angle θ (or 2θ). The acqui-

red data is then processed by the Rietveld method in order to minimize the residual function using a non-linear least squares algorithm. With that we can then refine the crystal structure of a compound. XRPD is typically used to determine the occurrence of a non-crystalline solid form, since it can be determined by observing the loss of distinct XRPD peaks characteristic of crystalline order, and the appearance of a general »halo« pattern (1). There are, however, a number of different non-crystalline phases that can give broad halos in the measured XRPD data, the most commonly observed of which are super-cooled liquids and glasses (96). Grinding or milling crystals can remove all traces of crystalinity according to XRPD. Cryo-grinding studies provide an ideal experimental approach to investigate the formation of amorphous material and the nature of the X-ray diffraction response. The typical behavior observed when grinding a crystalline organic material to produce amorphous material is that an increasing percentage of the crystalline material will collapse to amorphous as a function of grinding time. The amorphous local packing generates broad halos in the XRPD pattern that are not correlated to the crystalline peaks. If no significant change is observed in the crystalline diffraction peaks upon grinding, the ground sample can be modeled as a phase separated binary mixture of thermodynamic amorphous and crystalline materials. Although successive micronization should eventually lead to an amorphous structure, a possibility exists that the material has achieved a microcrystalline state, containing crystals so small that they pass the detection of XRD. Johari *et al*. (97) used DSC to distinguish between amorphous and microcrystalline states based on the presence or absence of glass transition when XRD failed to do so. It should also be noted that materials ground to or exhibiting the same X-ray amorphous pattern might crystallize at different times or to different crystalline forms. This can be perceived as a lack of control, resulting in amorphous materials that are not consistent. A better understanding of short-range and long-range interactions in amorphous materials using atomic pairwise distributions can help explain and possibly control the physical stability of materials. Data analysis of X-ray amorphous patterns plays a significant role in addressing the primary regulatory concerns for drug commercialization: understanding, stability and reproducibility (98).

Atomic pairwise distribution

The atomic pairwise distribution function (PDF) is a method used to analyze the local structure based on the total scattering pattern from the crystalline, nanocrystalline, quasi crystalline or amorphous materials (99, 100). The technique uses the Fourier transformation of XRPD diffractograms to produce a trace in the coordinate system. The y-axis in the PDF graph corresponds to the probability of finding two atoms separated by a distance plotted on the x-axis. In the material science community the PDF technique has been used for several decades (101–105) and has more recently been applied by the pharmaceutical community to study short- and long-range order amorphous glassy materials (106–111). It has been used in studies to investigate crystalline defects, help in the crystal structure determination (106, 107, 110), characterization of polymer/drug systems (111–113) and the use of multivariate data analysis has alleviated the interpretation of PDF (108, 111). It could be a possible route to gain a deeper insight into the degree of disorder in a milled sample. This is due to the fact that PDF displays the probability of finding two atoms separated by a given distance (99). Therefore, it could be expected that with increasing disorder in the sample, the signal amplitude of the PDF trace would

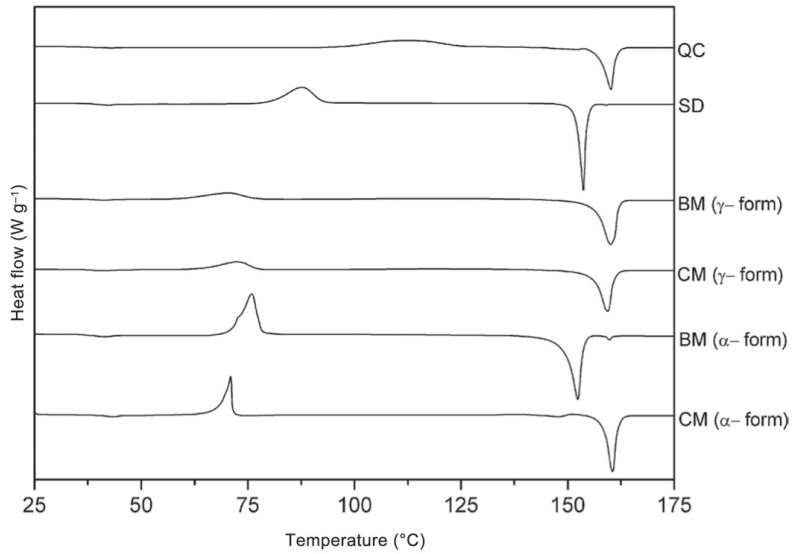

Fig. 6. DSC curves of a freshly prepared amorphous form of indomethacin prepared by different preparative techniques. Key: QC – quench cooled, SD – spray dried, BM – ball milled, CM – cryo-milled. Adapted from ref. 8 with permission.

be further reduced, until the highest possible disorder for a given cryo-milling process has been achieved. Bøtker *et al.* (81) have found that PDF is capable of assessing the minimal cryo-milling time that facilitates the highest degree of disorder and stability in cryo-milled samples of indomethacin.

Differential scanning calorimetry

Thermal methods have been used for amorphous content determination. DSC is a method commonly used for investigation of phase behavior of pharmaceutical solids, including quantification of the amorphous content (112). Depending on the instrument type, measuring parameters and experimental conditions, there are several methodologies that can be employed to determine the latter. Among them are conventional DSC, modulated temperature DSC (MTDSC) and hyper- or high-speed DSC (HSDSC) (113). Conventional DSC is based on a linear heating rate. In temperature modulated DSC (TMDSC), a small sinusoidal temperature modulation is applied to the sample in addition to the usual linear ramp. In the newest technique, hyper-DSC, controlled fast heating and cooling rates of 50 up to 500 °C min^{-1} are used (114). This significantly increases measurement sensitivity because the increased scan rate leads to higher heat flow. Whereas amorphous character can be difficult to detect in highly crystalline solids using the conventional DSC technique, hyper DSC can show glass transitions with enhanced sensitivity and a less time (114).

As mentioned before, amorphous materials exist as solid or liquid glasses like rubbers. The transition between these states is a second-order change phase, which occurs at T_g (115). There are at least three ways to determine T_g. Standard T_g is the temperature corresponding to the point on the heat flow curve where the specific heat change is 50 %

Table II. Thermal properties of amorphous forms of indomethacin prepared by different preparative techniques (8)

Technique	T_g (°C)	C_p at T_g (J g^{-1} K^{-1})	Onset of crystallisation (°C)	ΔH_{relax} (J g^{-1})
QC	42.15 ± 1.16	0.50 ± 0.003	96.93 ± 1.02	1.03 ± 0.26
SD	41.25 ± 0.28	0.47 ± 0.28	73.59 ± 7.18	0.68 ± 0.31
BM (γ-form)	39.23 ± 2.19	0.57 ± 0.03	62.14 ± 4.16	0.28 ± 0.09
CM (γ-form)	40.27 ± 3.57	0.52 ± 0.16	60.84 ± 2.91	0.95 ± 0.84
BM (α-form)	37.92 ± 2.02	0.70 ± 0.09	70.16 ± 0.70	0.84 ± 0.43

Adapted from ref. 8 with permission.

of the change in complete transition. This is the temperature at which heat capacity is midway between the liquid and glassy state (116). Glass transition can be also regarded as the inflection point of the DSC curve associated with glass transition. If a high relaxation peak follows glass transition, the inflection point of the DSC curve can differ from the real inflection point of the glass transition. Fictive temperature refers to the point on the enthalpy curve where the change of slope occurs (117). The enthalpy curve is the integral of the specific heat curve (Fig. 6). Fictive temperature is the intersection of the extrapolated pre-transition and post-transition baselines on the enthalpy curve.

ΔC_p is linearly proportional to the amorphous content in case that amorphous glasses are in the same state. The largest change in specific heat is equal to the difference between crystalline and rubber states. When glass transition is used for the quantification of amorphous content, there has to be a reference material. The starting point for the development of this method is to ensure that the change in specific heat of a 100 % amorphous sample is reproducible. Many things influence ΔC_p. Glasses are known to change their properties when annealed below their glass transition temperature. The release of relaxation enthalpy that follows glass transition corresponds to the enthalpy difference between annealed and quenched (non-annealed) glass (118). Karmwar *et al.* (8) have shown that DSC curves of amorphous forms of indomethacin prepared by quench cooling and spray drying exhibit different T_g as shown in Fig. 6 and Table II.

Solution calorimetry

Few publications have utilized solution calorimetry to determine the extent of drug and excipient crystallinity (119–123). This is a thermal technique in which the temperature change produced by a chemical or physical interaction during the mixing of two solutions or of a solid or a liquid in a constant temperature environment is monitored as a function of time (120). Studies suggest that solution calorimetry can be used to determine the amorphous content of a drug and excipient, when the solubility and dissolution rate of the compound in the chosen solvent are reasonably high. Typically, 100 % crystalline and 100 % amorphous materials have been physically mixed to prepare samples of varying percent crystallinities, and a linear relationship between the heat of solution and the mass percent crystalline fraction present in the resulting mixture has been

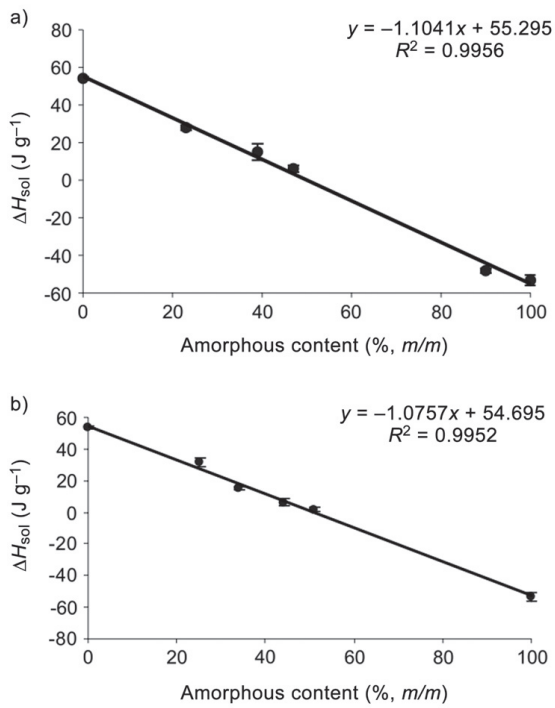

Fig. 7. Relationship between the $\Delta_{sol}H$ and the amorphous content of: a) physical mixtures; b) spray-dried samples. Mean values SD are shown (n = 4). Adapted from ref. 124 with permission.

demonstrated (120, 121). Harjunen *et al.* (124) have shown that this method can be also used for assessment of the amorphous content of lactose that was not completely dissolved in a solvent. An excellent correlation was observed between the enthalpy of solution ($\Delta_{sol}H$) in water and the amorphous content of the samples, as shown in Fig. 7. Further, there was also a linear correlation between the enthalpy accompanied with addition of a lactose sample to an oversaturated aqueous solution ($\Delta_{sat}H$) and the amorphous content of the samples, as shown in Fig. 8. Interestingly, linear relationship was observed between the $\Delta_{sat}H$ and the $\Delta_{sol}H$ of the samples. Therefore, solution calorimetry may represent a rapid and simple method for determining the amorphous content also in samples that are not completely dissolved in the solvent.

Density measurements

Solid density is a physical property, the value of which is frequently required in both fundamental and applied pharmaceutics. True density may be obtained using pycnometry, flotation density measurements, or from a single crystal structure. Flotation density measurement, however, is not suitable for powder mixtures (125, 126). Crystalline materials in general have higher density than their amorphous counterparts because the atoms in the crystal lattice are located at a minimum possible distance from each other. An increase in lattice disorder (decreasing crystallinity) usually results in an increase in volume and therefore a decrease in density. Changes in crystalinity should therefore be accompanied by gradual, progressive changes in density (127). The degree of

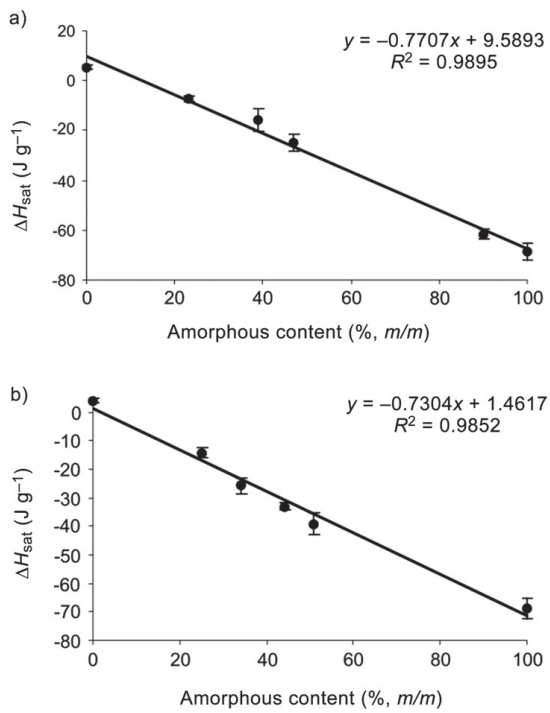

Fig. 8. Relationship between $\Delta_{sat}H$ and the amorphous content of a) physical mixtures, b) spray-dried samples. Mean values SD are shown (n = 4–8). Adapted from ref. 124 with permission.

crystallinity of a sample can be determined by *Eq.* (2) (ρ – obtained density, ρ_a – density of the fully amorphous state, ρ_c – density of the crystalline state):

$$\text{Crystallinity} = \frac{\rho - \rho_a}{\rho_c - \rho_a} \times 100 \qquad (2)$$

Different density measurement techniques were used in literature to detect low levels of the amorphous phase in crystalline pharmaceuticals (128) or to determine sample crystallinity (1, 129). Therefore, density measurements can be also used as an alternative technique to determine the solid state of pharmaceuticals. Salekigerhardt *et al.* (130) have shown that an increase in density correlates with the disorder of solid sucrose (Fig. 9).

Gravimetric vapor sorption

Gravimetric vapor sorption is a technique used to determine the vapor sorption isotherms. Instruments measure how the mass of the sample changes as the vapor environment surrounding the sample is altered. An increase in mass is typically associated with vapor sorption, whilst the occurence of mass decrease is attributed to vapor desorption. Mixed saturated and dry carrier gas streams are used to control the vapour concentration around the sample. Dynamic vapor sorption has been previously used to characterize amorphous or partially amorphous systems (131). The principle behind it is that

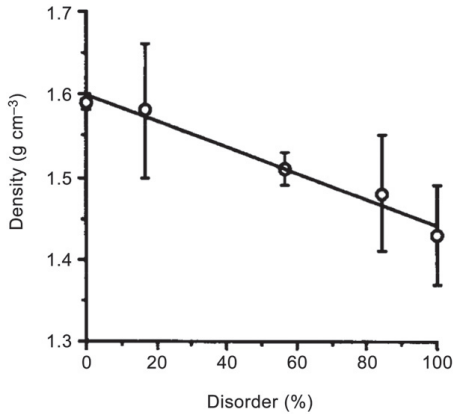

Fig. 9. Density *vs*. percent disorder for mixtures of amorphous and crystalline sucrose. Adapted from ref. 130 with permission.

amorphous materials typically have a higher surface area and vapour affinity than their crystalline counterparts. More recently Vollenbroek *et al.* (132) have developed a method which is based on gravimetric vapor sorption that allows determination of the amorphous lactose content over the range of 0.1–100 %. However, it must be noted that using dynamic vapor sorption for determination of amorphous content may be flawed, because direct comparison of partially amorphous particles to wholly amorphous and wholly crystalline systems may result in a significantly different outcome. This is due to the fact that semicrystalline materials have different molecular mobility compared to wholly amorphous or crystalline material (13).

Inverse gas cromatography

Inverse gas chromatography (IGC) is a vapor sorption technique in which powder is packed in a column and known vapors (usually at infinitine dilution in a carrier gas) are injected. It provides access to several physicochemical (surface and bulk) properties of materials, including their surface energy, phase transitions, solubility parameter, crystallinity, and acid-base characteristics (133, 134). It has been also used to detect surface energy changes caused by milling (135). It was found for sucrose that milling does not influence the crystal structure particles, but only the particle size and relative exposure of specific crystal planes. From the retention times of the probes it is possible to assess the surface nature of the material in the column (130). It is expected that this vapor sorption approach is also able to detect small amounts of amorphous content (88). Planinsek *et al.* (136) have shown that IGC is an efficient method for the quantification of the fraction of amorphous surface of milled indomethacin. It was shown that combination of IGC with DSC enables not only quantification but also localization of structural changes of milled indomethacin. Namely, it enables differentiation between the transformed structure at the surface of particles and transformations of the bulk region.

MID infrared spectroscopy

Middle (MID) infrared methods can reflect significant spectral differences between crystalline and amorphous phases and are hence used to quantify the crystalline content, since the intensity of the vibrational bands is directly proportional to the concentration of the concerned phase. Amorphous form of a given drug give rise to IR spectra that differs from its crystalline counterparts. The origin of these differences relates to both the wider range of conformations typically present in an amorphous solid, which normally leads to the presence of broader peaks relative to those found in the crystalline spectrum, as well as to differences in intermolecular interactions (1). Infrared procedures for measuring the degree of crystallinity are based upon the measurement of the intensity of a peak, characteristic of the crystalline state with reference to a peak that is independent of the crystal state of the substance. Nakai *et al.* (75) have shown that it is possible to study the effect of grinding on the crystallinity of microcrystalline cellulose (MCC) using the infrared technique and similarly Otsuka and his coworkers (137) also studied the effect of grinding on the crystallinity of cephalothin. MID-IR has also been found to be extremely useful to study amorphous solid dispersions (138). A study by Tang *et al.* (139) used FT-IR spectroscopy to characterize the differences between crystalline and the amorphous phases of dihydropyridine calcium channel blockers. For all compounds, the amorphous and crystalline samples gave rise to different spectra.

Near infrared spectroscopy

Near infrared spectroscopy (NIR) is a noninvasive technique, which requires no sample preparation; it is also non-destructive, enabling complete sample retrieval especially if used with the diffuse reflectance option. In addition, further quantitative information can be extracted from the data using chemoinfometrics, where the collected data is processed by means of statistical and applied mathematical techniques. Quantification of crystallinity is usually performed using the first (140) or second derivate spectra (95, 141, 142). Physical and chemical information such as polymorphism (143) and mutarotation (144) may be obtained. Buckton *et al.* (145) have shown that it is possible to monitor the crystallization of amorphous lactose in real time through examination of NIR spectra at certain wavelengths. Moreover, Hogan *et al.* (144) have shown that it is possible to quantify the amorphous content of lactose with NIR. Luner *et al.* (141) used the technique to determine the crystallinity of several pharmaceuticals, including indomethacin, lactose, ampicillin and sucrose. NIR was also used by Otsuka *et al.* (146) to monitor the stability of amorphous indomethacin in humidity controlled 96-well plates. Furthermore, Otsuka *et al.* (147) have shown that the crystallinity of unknown samples obtained by FT-NIR chemoinfometrical spectrometry was consistent with that obtained by conventional X-ray powder diffractometry and was more accurate (Fig. 10). According to the change in NIR absorbance of indomethacin, the solid structure of amorphous indomethacin was significantly different from that of the crystalline form.

Raman spectroscopy

Raman spectroscopy is a spectroscopic method used to observe vibrational, rotational, and other low-frequency modes in a system. It probes properties of the molecule it-

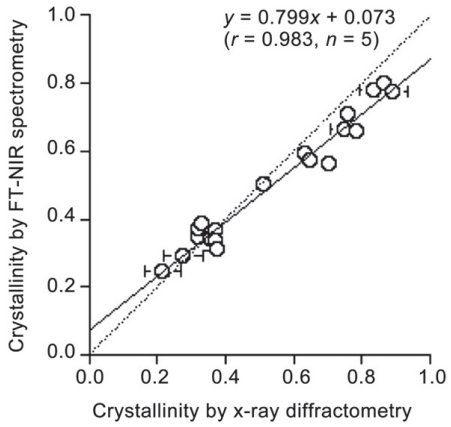

Fig. 10. Relation between predicted crystallinity of unknown indomethacin samples obtained by the conventional X-ray powder and FT-NIR method. Bars represent standard deviation. Adapted from ref. 147 with permission.

self, and changes in the solid-state properties of a substance are inferred from changes in the molecular conformation and molecular environment. This is due to the different packing conditions of the molecules in different solid forms. Differences can then be seen as subtle changes in the peak positions and intensities in the Raman spectra (148). It has been recently used in various studies for means of differentiation between differently prepared amorphous forms of the same substace. Karmwar *et al*. (8, 149) have shown that this is a suitable method for the detection of differences between differently prepared amorphous forms of indomethacin and simvastatin. Similarly Zimper *et al.* (79) have shown that Raman spectroscopy combined with multivariate analysis can detect and quantify disorder in a substance. Nevertheless, the authors point out that Raman spectroscopy, as a molecular level technique, is sensitive to the near order of solid materials and therefore could 'underestimate' the degree of disorder if the material remains near the range ordered to a certain extent (*i.e.*, exists as a dimer) in the amorphous state. However, in contrast to the findings of the previous authors, Boetker *et al.* (150) have found that it is unable to distinguish between differently prepared samples of amlopidine.

Dissolution tests

As the molecular mobility of the amorphous form is higher compared to the equivalent crystalline form, it may have an enhanced dissolution rate. This difference can be used to estimate the degree of amorphous content in a given sample. Although the amorphous form will have a higher dissolution rate because of high-surface free energy, there is an inherent risk of devitrification in the dissolution fluid (151), rendering dissolution tests useless for characterization of the solid state. However, the amount dissolved has been used to quantify crystallinity in the case of amorphous solid dispersions. (152). The main problem encountered in this technique is the effect of surface area; if not controlled stringently, it can significantly affect the results. To control the surface area, the powder is compressed. This may lead to probable phase changes. Also, the dissolution medium needs to be carefully selected as the components of the medium can influ-

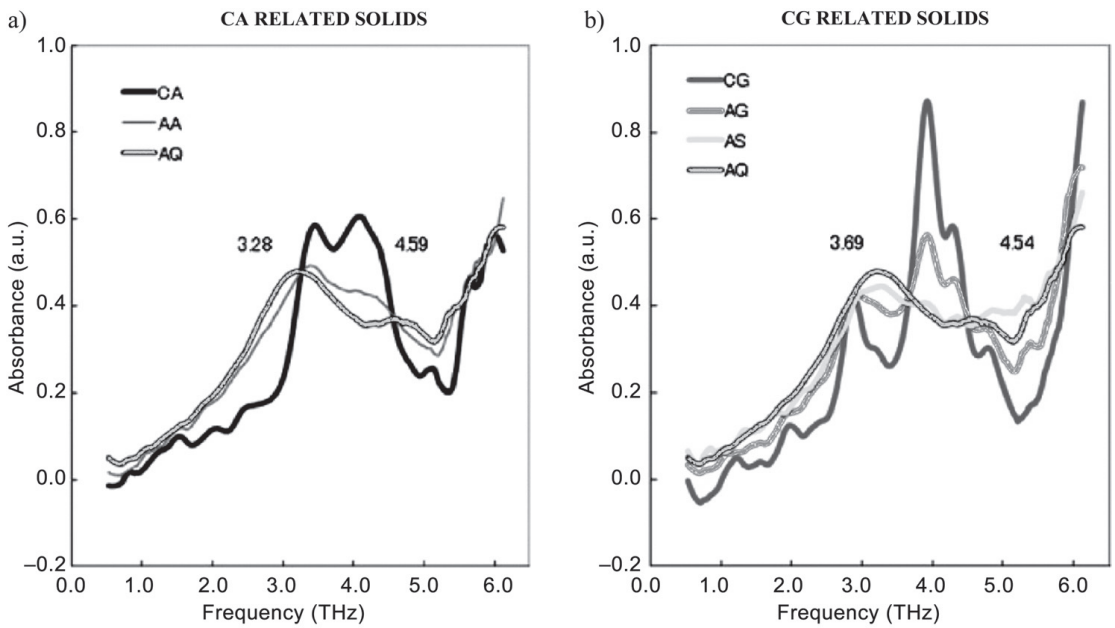

Fig. 11. Terahertz spectra of amorphous samples derived from α- and γ-indomethacin forms using different preparation techniques: a) powder samples prepared from α-indomethacin; b) powder samples prepared form γ-indomethacin. CA α-indomethacin, CG – γ-indomethacin, AA – ground amorphous α-indomethacin, AQ – fast cooled amorphous indomethacin, AG – ground amorphous γ-indomethacin, AS – slowly cooled indomethacin. Adapted from ref. (9) with permission.

ence the final outcome (88). Care must be taken when using this method if the transformation process includes extensive crystal defect formation, which is especially expected in the early stages of amorphization due to mechanical milling. Because the dissolution depends on solvent accessible surface and surface energy, crystal defect formation and increased surface could lead to false results.

Microcalorimetry

Microcalorimetry is a technique that has attracted much interest among pharmaceutical researchers as it can be used for various studies of preformulation. One among the possible uses of microcalorimetry is also assessment of the amorphous content (153–156). Determination of the amorphous content by microcalorimetry is based on the fact that conversion from the amorphous to the crystalline form is detected as an exothermic heat flow. The area under the exothermic peak is then proportional to the amorphous content. In some cases, the sensitivity of the calorimetric approach was even proven to be better than XRPD. Furthermore, one of the advantages of the technique is that it allows to measure the »real time« response in the calorimeter monitoring recrystallization of the amorphous substance (154).

Terahertz spectroscopy

Detection of terahertz radiation waves (THz), which have a frequency between 0.1 and 10 THz, is potentially very useful in probing intermolecular-level long range without sample contact and destructive treatment when characterizing solid-state materials, since it can induce low frequency bond vibrations, crystalline phonon vibrations hydrogen-bonding stretches, and torsion vibrations (153). A recent study by Otsuka *et al.* (9) has shown that THz spectroscopy is suited as a discrimination method between different amorphous states of pharmaceuticals called »poly-amorphous solids«. Fig. 11 shows the THz spectra of polymorphic crystalline forms and the amorphous solids of indomethacin obtained by the transmittance method.

CONCLUSIONS

Due to their higher solubility and bioavailability compared to the crystalline state, amorphous solids have received a lot of attention in the past decades. In this review, we have presented the possible methods of preparation of amorphous solids and their characterization. We elaborated on the background mechanisms involved in the process of amorphization, with the emphasis on amorphization through milling. The wide array of solid-state characterization techniques that can be utilized to investigate the different properties of the amorphous forms of substances prepared by various methods is also presented, since amorphous forms prepared by different means of amorphization often exhibit differences in stability and solubility. Moreover, differences can be also screened by commonly used methods of solid state characterization. We concluded that a multivariate visualization combined with complementing spectroscopic characterization offers an effective tool to screen for differences in amorphous samples.

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