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The different physical forms of a compound can exhibit pronounced differences in physicochemical properties. The term “physical form” encompasses polymorphs, solvates (also referred to as solvatomorphs), the non-crystalline (amorphous) form, and partially crystalline forms. Although numerous analytical techniques are available to characterize the physical forms of pharmaceutical compounds, this chapter will focus on thermal and crystallographic characterization techniques. While discussing the analytical techniques and their applications, we will use the term “polymorphism” in its broadest sense, with the understanding that with appropriate modifications, the techniques can often be extended for the characterization of the other physical forms of pharmaceuticals. Although crystallography (through crystal structure) provides the most definitive evidence of polymorphism, thermoanalytical, spectroscopic, and microscopic techniques are often used in a complementary fashion for the characterization of polymorphs.

Comprehensive and in-depth discussions of different thermoanalytical techniques can be found in the literature (1,2), and this chapter will specifically discuss the applications of thermoanalytical and crystallographic techniques in the characterization of pharmaceuticals. The specific thermoanalytical techniques to be discussed are differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and hot-stage microscopy (HSM). Several recent reviews and book chapters have covered this topic, and the examples presented are designed to complement the information in the literature (1–4). In the second part of the chapter, applications of X-ray powder diffractometry (XRD) (5,6) are provided. Finally, applications of variable-temperature XRD (VT-XRD) are discussed, specifically to monitor phase transitions as a function of temperature.

THERMOANALYTICAL TECHNIQUES

Thermal analysis methods can be conveniently defined as those techniques in which some property of the analyte is measured as a function of an externally applied temperature. In most applications, one effects a linear increase in the temperature of the sample while the property of interest is recorded on a continuous basis. Thermoanalytical methods are used to monitor endothermic processes (i.e., melting, boiling,

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sublimation, vaporization, desolvation, solid–solid phase transitions, and chemical degradation) as well as exothermic processes (i.e., crystallization and oxidative decomposition). Thermal methods are routinely used for the characterization of polymorphs, and in light of the experimental simplicity, often constitute the first line of techniques used to understand the polymorphic space of a system.

Differential Scanning Calorimetry (DSC)

In DSC, which is the most widely used technique for thermal characterization of pharmaceuticals, the analyte is subjected to a controlled temperature program and the temperature and the heat flow associated with a thermally induced transition is measured.

DSC has been developed along two lines: power-compensation DSC and heat-flux DSC. In power-compensation DSC, separate heating elements are in contact with the sample and reference cells, with the rate of heating by these elements being controlled and measured. Thus the sample and reference are maintained at the same temperature and the heat flow required to accomplish this is measured. The other methodology is that of heat-flux DSC, where the sample and reference cells are heated by the same element, and one monitors the direction and magnitude of the heat being transferred between the two.

A detailed theory of DSC is outside the scope of this chapter and has been discussed elsewhere (1,2). Briefly, the basic principle behind a change in the baseline of a typical DSC plot (power vs. temperature), signifying a thermal event, is a change in the heat capacity of the system under investigation. The basic equation (4) describing the relationship between the heat flux (Q) associated with a thermal event and the heat capacity (C_p) of the system under investigation is given by:

$$(dQ/dt) = C_p(dT/dt) \quad (1)$$

where dQ/dt represents power at the constant heating or cooling rate, dT/dt . DSC plots are obtained as the differential rate of heating [in units of watts (joules/second) or calories/second] against temperature, and thus represent direct measures of the heat capacity of the sample. The area under a DSC peak is directly proportional to the heat absorbed or evolved by the thermal event, and integration of these peak areas yields the heat of reaction (in units of calories/seconds · gram or joules/seconds · gram).

The effectiveness of DSC to detect various polymorphic forms is illustrated in Figure 1 (7). Profiles 1, 2, and 3 (*from top*) show the reversible transitions between the enantiotropically related forms I and III. The fourth DSC curve shows the melting of form II followed by crystallization and melting of form I. The bottom DSC profile is a heating curve of a mixture of forms II and III, which shows the respective melting endotherms but no transition to form I. This was tentatively attributed to the absence of form I seeds in the mixture of forms II and III, which were concomitantly crystallized as opposed to the pure forms (profiles 1–4), which were crystallized separately using different methods.

Standard Reference Materials (SRMs) are available from the National Institute of Standards and Technology for calibration of temperature and enthalpy of fusion for differential scanning calorimeters. Indium (SRM 2232; melting temperature = 156.6°C; enthalpy of fusion = 28.5 J/g), tin (SRM 2220; melting point = 232.0°C; enthalpy of fusion = 60.2 J/g), and gallium (SRM 2234; melting temperature = 29.8°C;

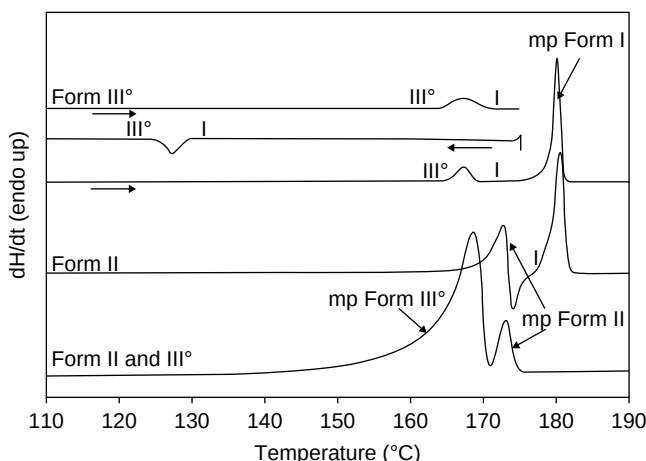


FIGURE 1 DSC profiles of cinacalcet hydrochloride polymorphs. The first (heating), second (cooling), and third (heating) profiles (*from top*) show the solid–solid transition (i.e., III° → I); the fourth profile shows melting of form II followed by crystallization and melting of form I; the fifth profile is a heating curve of a mixture of form II and form III° showing respective melting endotherms but no transition to form I signifying absence of form I seeds. *Note:* The direction of endotherm is *up*. *Source:* From Ref. (7).

enthalpy of fusion = 80.1 J/g) are suitable as calibration standards over the range of temperatures of interest in pharmaceutical analyses. Because DSC is also used at sub-ambient temperatures for the characterization of frozen solutions, mercury (SRM 2225; melting temperature = -38.9°C ; enthalpy of fusion = 11.5 J/g) is a suitable calibration standard. Heat capacity calibration in a DSC is usually carried out with synthetic sapphire (SRM 720), copper (SRM RM5), polystyrene (SRM 705a), or molybdenum (SRM 781D2).

Depending on the protocol used for temperature programming, a DSC experiment could be classified as being conventional, modulated, or accelerated (also known as Hyper-DSC®). In the conventional mode, the heating rate is constant and typically ranges from $0.1^{\circ}\text{C}/\text{min}$ to $20^{\circ}\text{C}/\text{min}$. The majority of DSC experiments are carried out in the conventional mode and may require optimization of the sample pan configuration (discussed later) and the experimental parameters to obtain the desired information (3). In Table 1, the effects of operational parameters are outlined.

In modulated DSC (MDSC), a changing (modulated) heating rate is superimposed on a constant average heating rate. The modulation is described by a frequency, determined by specific amplitude ($\pm^{\circ}\text{C}$) and period (seconds), such as $\pm 0.5^{\circ}\text{C}$ every 60 seconds, with the choice of the amplitude and period being dictated by the nature of the transition (8–10). The advantage of MDSC over conventional DSC is that it can separate the reversible heat flow from the non-reversible heat flow (Fig. 2). Reversible heat flow corresponds to events that respond to the changing heating rate (e.g., heat capacity change, melting). On the other hand, non-reversible heat flow events (e.g., crystallization or decomposition) are not affected by changing heating rates.

TABLE 1 Influence of Operational Parameters on the Outcome of DSC Experimental Results

	Effects
Sample size	
Large	Advantages (i) Non-homogeneous samples (ii) Detects “weak” transitions Disadvantages (i) Broad peaks (ii) Temperature of the event may be inaccurate (iii) Poor resolution (iv) Requires slow heating rate
Small	Advantages (i) Sharp peaks (ii) Increased resolution (iii) Permits faster heating rate Disadvantages (i) Decreased sensitivity
Heating rate	
Fast	Advantages (i) Increased sensitivity Disadvantages (i) Decreased resolution (ii) Decreased temperature accuracy
Slow	Advantages (i) Increased resolution Disadvantages (i) Decreased sensitivity

Source: Adapted from Ref. (2).

MDSC has found limited utility in the characterization of polymorphs (11,12). However, the inherent capability of this technique to separate overlapping melting and crystallization events has been demonstrated for the polymorphs of tristearin (13,14). MDSC is especially suited for the characterization of amorphous materials (15,16), and has enabled the quantification of amorphous content in a partially crystalline material (17). Because crystallinity differences could affect material performance, it is important that analysts are aware of low levels of lattice disorder in highly crystalline materials that were brought about by processing and storage conditions. Saklatvala et al. investigated two batches of an investigational crystalline drug, which showed pronounced differences in their stability and water sorption profiles, and MDSC enabled detection and quantification of the amorphous content in the batch characterized by the lower chemical stability and the increased propensity to take up water (18).

Hyper-DSC[®] uses accelerated heating rates (up to 500°C/min) in order to selectively investigate phase transitions. The high heating rates result in selective inhibition of transitions that would normally take place over the longer timescales

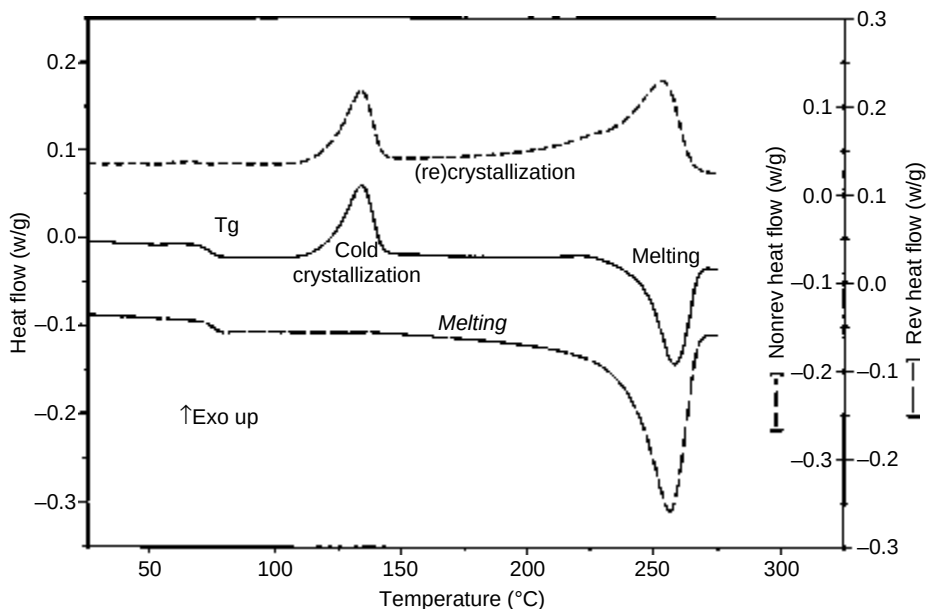


FIGURE 2 Modulated DSC profiles of quenched polyethylene terephthalate, showing the non-reversible (*uppermost trace*), total (*middle trace*), and reversible (*lowest trace*) heat flow curves. The exothermic recrystallization event is hardly discernible in the total heat flow but is prominent in the non-reversible heat flow. *Source:* From Ref. (66).

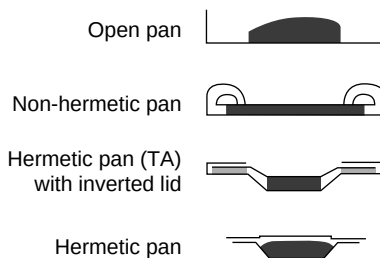


FIGURE 3 Diagrammatic representation of different DSC pan configurations. *Source:* From Ref. (9).

of conventional heating rates. A case in point are the polymorphs of carbamazepine, where a heating rate of 250°C/min was used to prevent simultaneous crystallization of form I during the melting of form III, thereby allowing determination of the enthalpy of fusion of form III (19).

In a typical DSC application, the sample is encapsulated in a pan and the lid is crimped, where the lid can be crimped either in a non-hermetic or in a hermetic manner (Fig. 3). In the hermetically crimped pan, a pinhole can be made in the lid to relieve pressure during the acquisition of the DSC curve. To minimize the head space, the lid can be inverted and then hermetically crimped.

The pan configuration can influence the outcome of a DSC experiment. This is especially true for solvates, because the desolvation kinetics, as well as the physical form of the product phase, can be profoundly influenced by the sample environment. In an open pan, there is no physical barrier to the escape of liberated solvent, and the purge gas (typically nitrogen) will facilitate removal of the liberated solvent. The reasonably unrestricted nature of a non-hermetically crimped pan will also permit ready escape of the liberated solvent. At the other extreme, the liberated solvent cannot escape in a hermetically crimped pan. In a sealed pan with a pinhole, the rate of vapor loss will become appreciable only when the vapor pressure exceeds atmospheric pressure. Different pan configurations, which result in varied desolvation conditions, have been used to generate different polymorphs of anhydrous carbamazepine and trehalose from their respective hydrates (20,21).

Pressure DSC (PDSC) enables DSC experiments to be carried out at elevated pressures as high as 1000 psi. PDSC is useful for the separation of overlapping dehydration and vaporization events that are observed following the dehydration of hydrates. Although the temperature of dehydration is likely to be unaffected by pressure, the boiling temperature of water is pressure dependent and can be ascertained from the Clausius–Clapeyron equation. In addition, the potential interaction of the liberated water with the anhydrous product phase can also be ascertained. Han and Suryanarayanan (22) used PDSC to successfully resolve overlapping dehydration and vaporization transitions observed when carbamazepine dihydrate was heated (Fig. 4, *upper left panel*). The conventional DSC of carbamazepine exhibited two overlapping endotherms over the temperature range of 85°C to 100°C, attributed to dehydration followed by vaporization, which were followed by the melting of non-solvated carbamazepine around 189°C. When the pressure was increased, only a single endotherm attributable to dehydration was observed at approximately 90°C. Interestingly, several thermal events were observed between the dehydration and the melting temperatures.

A limitation of DSC is that although it reveals the existence of thermally induced transitions, the nature of these transitions can be difficult to determine. In an effort to simulate the PDSC experiments, Han and Suryanarayanan (22) heated carbamazepine dihydrate in a “sealed” environment. Following dehydration, the released water caused an increase in pressure by being retained in the holder, although the pressure was uncontrolled. As shown in the *upper right panel* of Figure 4, the carbamazepine polymorphic transitions were further characterized by VT-XRD. The formation of β -carbamazepine at $\sim 90^\circ\text{C}$ was evident in the diffraction patterns on the basis of peaks observed at scattering angles of 13.1, 15.3, and $18.7^\circ 2\theta$. Based on the disappearance of the characteristic peaks of carbamazepine dihydrate (at 8.9 and $12.3^\circ 2\theta$), dehydration appeared to be complete by 100°C . Around 160°C , several characteristic peaks of γ -carbamazepine were observed, indicating a $\beta \rightarrow \gamma$ solid–solid phase transition. Based on the decrease in the intensities of the β -carbamazepine peaks, and the increase in the intensities of the γ -carbamazepine peaks, the $\beta \rightarrow \gamma$ phase transition was accelerated as the temperature was increased to 180°C . These VT-XRD results aided in the interpretation of the DSC results. When DSC was conducted under elevated pressure, usually four endotherms were observed. The first endotherm at $\sim 90^\circ\text{C}$, as explained earlier, is attributed to dehydration. The two overlapping endotherms at $\sim 120^\circ\text{C}$ and 125°C are attributed to the $\beta \rightarrow \gamma$ carbamazepine phase transition and to the vaporization of water. The melting of γ -carbamazepine occurred at $\sim 189^\circ\text{C}$. This scheme is

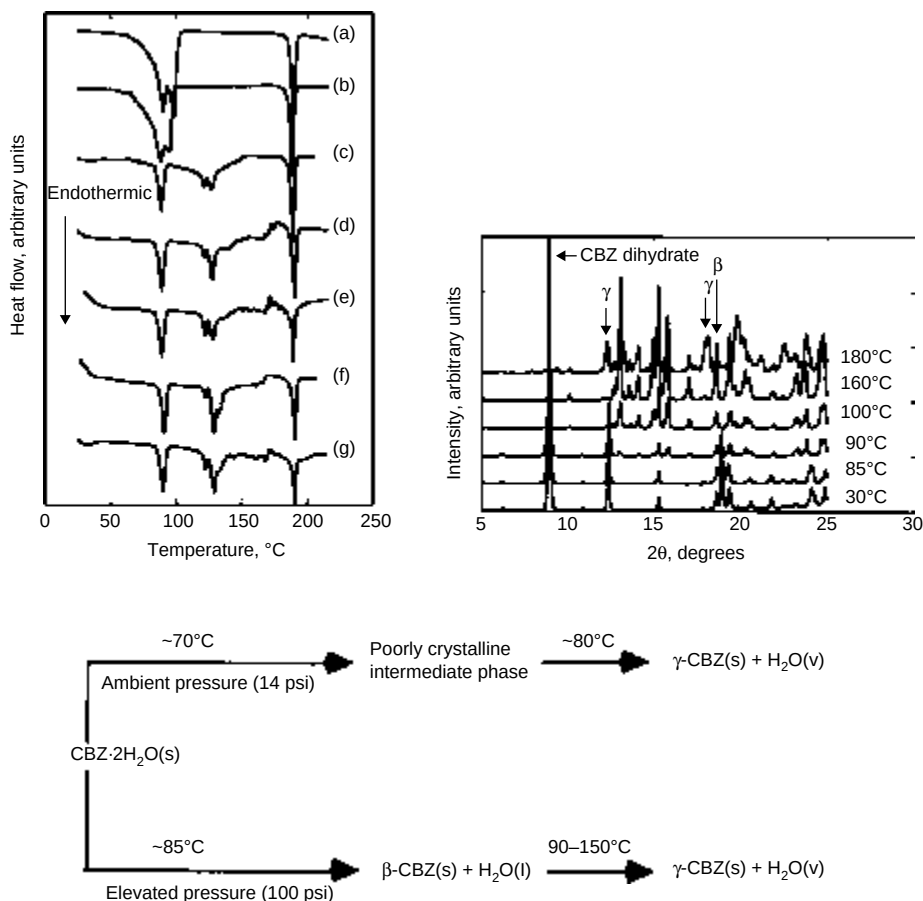


FIGURE 4 (Upper left panel) DSC of carbamazepine dihydrate at different pressures: (a) ambient pressure in conventional DSC cell, (b) ambient pressure in PDSC cell, (c) 100 psi, (d) 200 psi, (e) 300 psi, (f) 400 psi, (g) 600 psi. (Upper right panel) VT-XRD of carbamazepine dihydrate. Peaks characteristic of the dihydrate as well as anhydrous β and γ forms are shown. (Lower panel) Schematic showing carbamazepine dihydrate transitions under ambient and elevated pressures. Source: From Ref. (22).

illustrated in Figure 4 (lower panel) and shows the transitions of carbamazepine dihydrate at ambient and elevated pressures.

An emerging technique that could be considered as a variant of DSC is microthermal analysis, which combines the principles of DSC and scanning probe microscopy. Microthermal analysis was used to identify local regions of different polymorph content in a 50:50 mixture of two cimetidine polymorphs (23). This technique also has the potential to map a tablet surface for polymorph content uniformity.

Thermogravimetry

Thermogravimetry (TG, and also known as thermogravimetric analysis or TGA) is a technique where one uses a very sensitive balance to continuously determine the

weight of the analyte as a function of temperature or time. This simple technique provides valuable information that can aid in the identification and characterization of the thermal events associated with thermally induced reactions of solvates. For example, several hydrates of nedocromil sodium were identified and profiled using TG as one of the characterization tools (24). As the sample was heated, the weight loss occurred in a stepwise fashion, which facilitated calculation of the hydrate stoichiometries based on the percent weight loss at each step.

As discussed elsewhere in this book, there are different ways in which water can associate with solids. In authentic hydrates, water is incorporated in the crystal lattice, whereas in amorphous and partially crystalline materials, water can associate with disordered regions in the lattice. Water can also be adsorbed and also exist as "bulk water" in incompletely dried solids. TG analysis can also be used to distinguish between some of these states of water in solids. Compared with dehydration, desorption (adsorbed as well as bulk) typically occurs at lower temperatures, although these two processes can overlap. As shown in Figure 5, for carbamazepine dihydrate, when the TG experiment is carried out under isothermal conditions, there can be sequential loss of sorbed and lattice water (25).

TG analysis performed under isothermal conditions can be used to study the kinetics of phase transitions in solvatomorphs (26,27). Alkhamis et al. (26) carried out model-independent isoconversional analysis of the isothermal desolvation of the monohydrate and ethyl acetate solvate of fluconazole. One potential shortcoming of this technique is that a separate and direct technique might be required to confirm the cause of weight loss. In order to address this inadequacy, the TG instrument can be interfaced with additional instrumentation capable of identifying the vapor released upon heating. For example, TG has been used in combination with infrared absorption spectroscopy to understand the thermal behavior of a hydrate (28). In this study, two different thermal events resulting in weight loss were shown to be due to separate dehydration and decomposition processes.

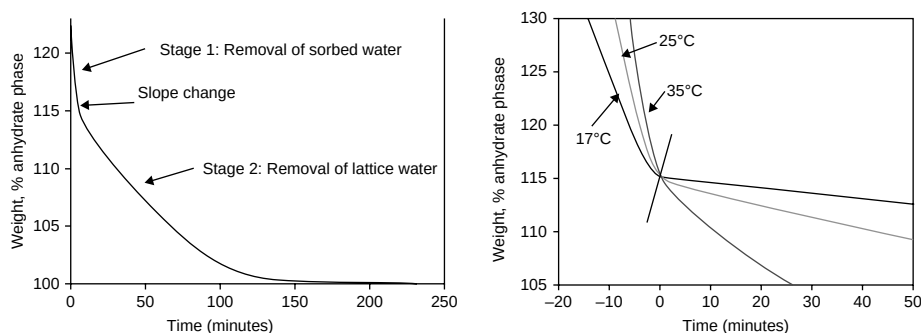


FIGURE 5 (Left panel) Dehydration of a wet sample of carbamazepine dihydrate at 0% RH (25°C). The plotted sample weight is based on the dry sample weight after complete dehydration. The first and second stages of dehydration are separated by the point of slope change. (Right panel) Effect of temperature on desorption and dehydration kinetics of carbamazepine dihydrate shown at three representative temperatures. For the purpose of comparison, the x-axis origin is the time point of transition from desorption to dehydration (point of slope change in left panel). Source: From Ref. (25).

Temperature calibration of TG instrumentation can be accomplished using melting point standards or the Curie point transition of a paramagnetic material (ASTM E1582) (29). Although not considered a “reference standard,” calcium oxalate monohydrate is frequently used to validate the performance of a TG system (30).

Hot-Stage Microscopy

Visualization of solids using a microscope often provides unique insights not afforded by other techniques. The visual observation of the analyte, placed on a temperature controlled stage (“hot stage”) of a microscope, forms the basis of HSM. For the characterization of polymorphs and solvates, HSM, with DSC and TG analyses, forms an optimal routine three-pronged approach. Vitez et al. (31) have briefly outlined the historical evolution of HSM from being merely a melting point determination tool to being an integral part of polymorph characterization. The practice and application of HSM has been reviewed and discussed in recent publications (3,32).

One of the primary advantages of HSM is the small amount of sample needed, which, given the limited availability of an active pharmaceutical ingredient (API) in the discovery phase, often represents a very important consideration. When characterizing solvates, the analyte is typically dispersed in silicone oil in order to aid in the visualization of the thermally induced release of any included solvent. As shown in Figure 6, a desolvation process is usually manifested by loss of transparency (perceived as a darkening of the crystals) (33), and in the formation of bubbles (specifically for hydrates) in the oil (34,35). Caution should be exercised in data interpretation because this observation could be potentially complicated by the release of entrapped air from the solids or by the simple presence of air bubbles in the dispersion medium. In addition, crystals may also darken as a result of decomposition.

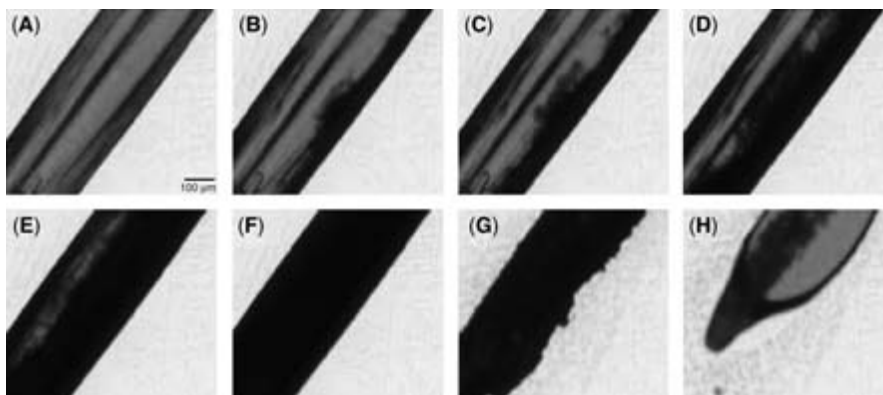


FIGURE 6 Hot-stage photomicrographs of toluene solvate of picryltoluidene, taken at various temperature values: (A) 25°C; (B) 35°C, onset of desolvation from crystal edges; (C) 45°C; (D) 55°C; (E) 65°C; (F) 80°C, completely desolvated anhydrous form; (G) 162.5°C, melting onset of the anhydrous form; (H) 163°C, remaining crystal of the anhydrous form in the melt. Source: From Ref. (33).

In some instances, desolvation may be accompanied by a change in crystal habit. For example, Tonder et al. used HSM to study the desolvation of different niclosamide solvates (36). Although the solvate crystals were characterized by different morphologies, desolvation resulted in the appearance of crystals having the same habit as that of the anhydrate form. In addition, the desolvated crystals were all observed to melt over the same temperature range as did the anhydrate, which was taken as confirming their transformation to the anhydrate phase.

Crystallization from the amorphous state can be demonstrated by the appearance of birefringence. In order to explain DSC observations, Bottom monitored the crystallization of a metastable spherulitic polymorph of sulfapyridine from the amorphous state through a solid-state phase transition to the stable needle-shaped form (12). Grooff et al. (37) used HSM as one of the tools to characterize crystallization of a metastable polymorph of nifedipine from the amorphous state. In this work, two other polymorphs were identified, and an enantiotropic relationship between two of the polymorphs was confirmed by direct observation of a reversible solid-state transition between the two crystal forms.

Microscope stage attachments are commercially available having a wide temperature range (less than -150°C to higher than 350°C) and excellent temperature stability ($\pm 0.1^{\circ}\text{C}$). In addition, most modern hot-stage systems are equipped with a high-resolution camera capable of rapid image collection, and which is controlled by a computer interface. All these accessories have drastically reduced the subjectivity associated with microscopy and have increased the efficiency of data collection. Consequently, HSM has evolved into one of the primary techniques that is used for the routine identification and characterization of pharmaceutical solids.

CRYSTALLOGRAPHIC CHARACTERIZATION TECHNIQUES

The technique of XRD is exceedingly important in the study of polymorphs and solvatomorphs because it represents the primary method whereby one can obtain fundamental information about the structure of a crystalline substance. The methodology is highly suited for the differentiation of crystal forms, as it would be only by pure coincidence that two compounds might form crystals having identical XRD patterns due to identical three-dimensional spacing of planes in all directions. One example of accidental isostructure is the trihydrate phases of ampicillin and amoxicillin (38), but such instances are uncommon. Typical applications of XRD methodology include the determination of crystal structures, evaluation of polymorphism and solvate structures, evaluation of degrees of crystallinity, and the study of phase transitions.

Every crystal consists of exceedingly small fundamental structural units, which are repeated indefinitely in all directions. In 1830, Hessel conducted a purely mathematical investigation of the possible types of symmetry for a solid figure bounded by planar faces, and deduced that only 32 symmetry groups were possible for such objects. The same conclusion was reached by Bravais in 1949, and Gadolin in 1867. These 32 crystallographic point groups are grouped into six crystal systems, denoted as triclinic, monoclinic, orthorhombic, tetragonal, trigonal, hexagonal, and cubic. Each crystal system is characterized by unique relationships existing among the crystal axes and the angles between these, and this information is summarized in Table 2.

TABLE 2 Characteristics of the Six Crystal Systems

System	Description
Cubic	Three axes of identical length (identified as a_1 , a_2 , and a_3) intersect at right angles.
Hexagonal	Four axes (three of which are identical in length, and denoted as a_1 , a_2 , and a_3) lie in a horizontal plane, and are inclined to one another at 120° . The fourth axis, c , is different in length from the others, and is perpendicular to the plane formed by the other three.
Tetragonal	Three axes (two of which are denoted as a_1 and a_2 , and are identical in length) intersect at right angles. The third axis, c , is different in length with respect to a_1 and a_2 .
Orthorhombic	Three axes of different lengths (denoted as a , b , and c) intersect at right angles. The choice of the vertical c axis is arbitrary.
Monoclinic	Three axes (denoted as a , b , and c) of unequal length intersect such that a and c lie at an oblique angle, and the b axis is perpendicular to the plane formed by the other two.
Triclinic	Three axes (denoted as a , b , and c) of unequal length intersect at three oblique angles.

Bragg (39) explained the diffraction of X rays by crystals using a model where the atoms of a crystal are regularly arranged in space, and that they can be regarded as lying in parallel sheets separated by a definite and defined distance. Then he showed that scattering centers arranged in a plane act like a mirror to X rays incident on them, so that constructive interference would occur in the direction of specular reflection. Within a given family of planes, defined by a Miller index of (hkl) and with an inter-planar distance d , each plane produces a specular reflectance of the incident beam. If the incident X rays are monochromatic (having wavelength equal to λ), then for an arbitrary glancing angle of θ , the reflections from successive planes are out of phase with one another. This yields destructive interference in the scattered beams. However, through the systematic variation of analysis angles, a set of values for θ can be found so that the path difference between X rays reflected by successive planes will be an integral number (n) of wavelengths, and then constructive interference would occur. One ultimately obtains the expression known as Bragg’s law that explains the phenomenon:

$$2d\sin \theta = n\lambda \tag{2}$$

Unlike the diffraction of light by a ruled grating, the diffraction of X rays by a crystalline solid leads to constructive interference (i.e., reflection) only at critical Bragg angles. When reflection does occur, it is stated that the plane in question is reflecting in the n th order, or that one observes n th order diffraction for that particular crystal plane. Therefore, one will observe an X-ray scattering response for every plane defined by a unique Miller index of (hkl).

Single-Crystal X-Ray Diffractometry

Single-crystal XRD involves an assignment of the arrangement of atoms based on an electron density map generated by diffraction of a pure, regular, and adequately large crystal (40). Structural information derived from an XRD study of a single

crystal is the most fundamental description of a polymorph or solvatomorph, and helps to explain its physical properties.

Generally, polymorphic structures can be classified into one of two main categories. The first is associated with molecules that can only exist as a rigid grouping of atoms that can be stacked in different motifs to occupy the points of different lattices (i.e., packing polymorphism). One of the best-known instances of packing polymorphism is the allotropic system of carbon, namely graphite and diamond. As shown in Figure 7, in diamonds each carbon atom is tetrahedrally surrounded by four equidistant neighbors, and the tetrahedra are arranged to give a cubic unit cell. Graphite is composed of planar hexagonal nets of carbon atoms, which can be arranged to yield either a hexagonal unit cell (the α -form) or a rhombohedral unit cell (the β -form).

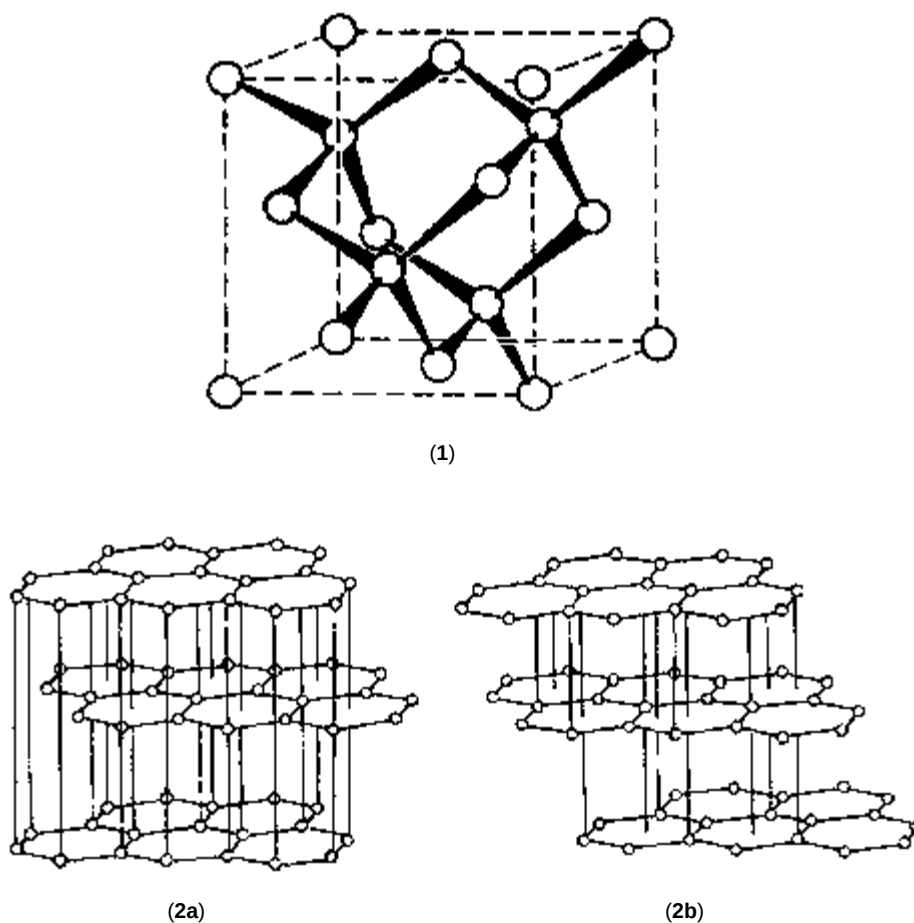


FIGURE 7 Crystal structure of (1) diamond, showing the tetrahedral coordination of each carbon atom. Also shown are the crystal structures of the two polymorphs of graphite, specifically (2a) the hexagonal α -form, and (2b) the rhombohedral β -form.

The other category is associated with molecules that are capable of existing in conformationally different arrangements, each of which can crystallize in its own characteristic structure. This latter behavior has been termed conformational polymorphism (41), and the probucol system represents an extreme example of a compound where the polymorphism arises from the packing of different conformers (42). Although both polymorphs were found to be monoclinic, the unit cells belonged to different space groups and the molecular conformations of the title compound were quite different (Fig. 8). In form II, the C–S–C–S–C chain is extended,

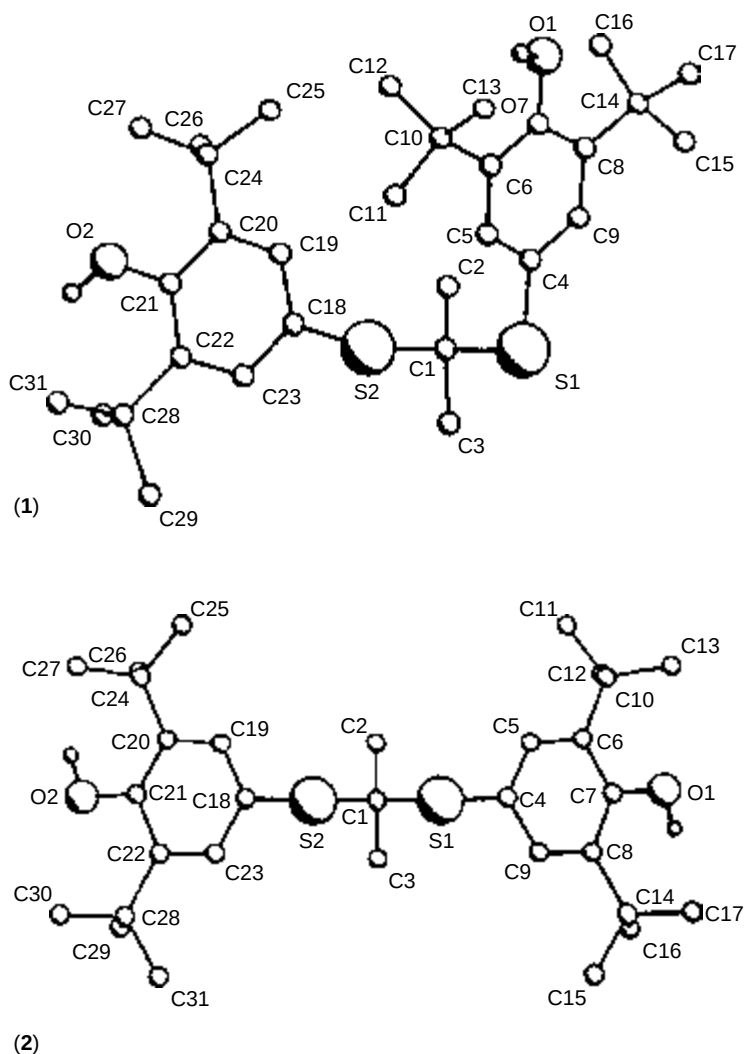


FIGURE 8 Conformation of the probucol molecule existing in **(1)** form I and in **(2)** form II. The figure was adapted from data contained in Ref. (42).

and the molecular symmetry approximates C_{2v} . This symmetry is lost in the structure of form I, where the torsion angles about the two C–S bonds deviate significantly from 180° . The extended conformer was shown to be less stable relative to the bent conformer, as simple grinding was sufficient to convert form II into form I.

Not all instances of conformational polymorphism are as dramatic as the probucol system, and often different conformers of a single sidechain are able to pack into different crystalline arrangements. For instance, the two polymorphs of *p*-(1*R*,3*S*)-3-thioanisoyl-1,2,2-trimethylcyclopentane carboxylic acid were found to be associated with different conformations of the carboxylate group (43). Torsion about a single C–N bond was shown to be the origin of the polymorphism detected for lomeridine dihydrochloride (44). Finally, relatively small differences in molecular conformation were detected for the two polymorphic and four solvated crystalline forms of spironlactone (45).

The analysis of single-crystal XRD data is divided into three parts (46–49). The first of these is the geometrical analysis, where one measures the exact spatial distribution of X-ray reflections and uses these to compute the size and shape of a unit cell. The second part entails a study of the intensities of the various reflections and uses this information to determine the atomic distribution within the unit cell. Finally, one looks at the X-ray diffraction pattern to deduce qualitative information about the quality of the crystal or the degree of order within the solid. This latter analysis may permit the adoption of certain assumptions that may aid in the solving of the crystalline structure.

A determination of the internal structure of a crystal requires the specification of the unit cell dimensions (axis lengths, and angles between these), and measurement of the intensities of the diffraction pattern of the crystal. For a given lattice, regardless of the content of the unit cell, the directions of reflection are the same. The experimental determination of these directions is used to deduce the reciprocal lattice of the crystal, which unambiguously yields the crystal lattice. In addition, the relative intensities diffracted by different planes depend on the contents of the unit cell. Their measurement leads to the determination of the crystal structure factor, and these data permit the determination of the atomic structure of crystals.

Crystallographic characterization of the physical form of an API may be used to understand its performance during pharmaceutical processing. One well-known case is that of polymorphic forms I and II of acetaminophen (also known as paracetamol). The crystal structures of both forms have been determined and the associated implications on their physical properties have been discussed (50). Monoclinic acetaminophen form I cannot be formulated into tablets by direct compression, but the presence of slip planes (illustrated in Fig. 9) in the orthorhombic form II allows its direct compression due to a plastic deformation process. This property is an immense advantage considering that direct compression is a cost-effective and time-efficient method of tablet production.

Crystallographic information is critical to understand dehydration of solvates that have channels in their crystal structures. As mentioned earlier, such solvatomorphs undergo desolvation under certain conditions, leading to the formation of isomorphic desolvates that tend to retain the three-dimensional structures of the parent compound (51,52).

Variable temperature and atmospheric-induced dehydration was characterized in form I of a small molecule developmental drug that was presented as a sodium salt and a trihydrate (53). It was shown that at 25°C , the compound lost 0.5% w/w

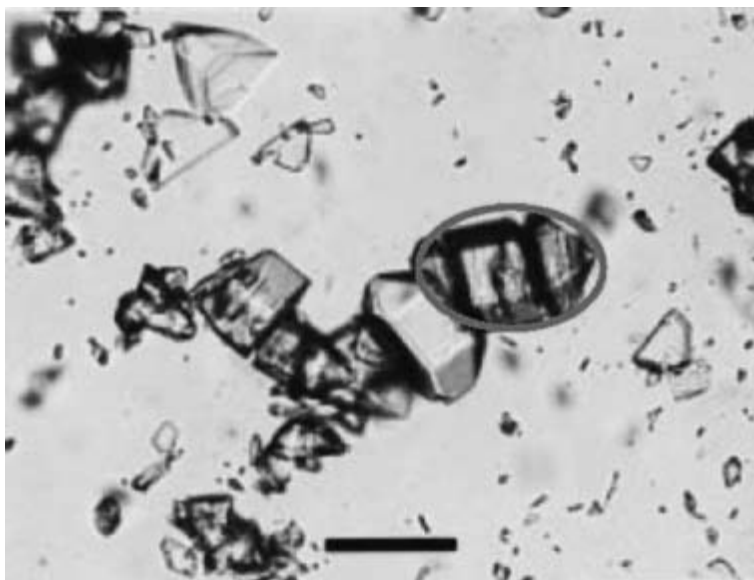


FIGURE 9 Pressure-induced formation of cleavages (*indicated by the ellipse*) in an acetaminophen form II crystal perpendicular to its length, showing the evidence of the existence of slip planes. *Source:* From Ref. (41).

water when the relative humidity was decreased from 90% to 15%, and that this water loss did not cause any change in the XRD pattern. A single-crystal XRD study revealed the existence of channels in the form I crystal structure (illustrated in Fig. 10), where one of the water molecules was weakly bound by hydrogen bonds and where the other two water molecules were bound to the sodium cation. Formation of the isomorphic dehydrate was attributed to the escape of some weakly bound water molecules through the channel, which did not compromise the integrity of the structure. Further decrease in relative humidity into the range of 5% to 15% resulted in a reversible phase transformation into form II, and at relative humidities below 5%, XRD showed that the compound transformed into the semi-crystalline form III. A combination of techniques was used to show that form II was formed as a result of the escape of both weakly and strongly bound water molecules, which caused a partial collapse of the channels. Further dehydration of the solid resulted in disordered anhydrate form III.

The interplay between intramolecular conformational flexibility and intermolecular hydrogen bonding may influence the properties of a polymorph (54), such as in the classic example of ritonavir (55). In 1998, the commercial form of this drug substance (tradename of Norvir®, and marketed as a semi-solid formulation contained in capsules) had to be reformulated because the newer lots failed to meet established dissolution specifications. This was attributed to the sudden appearance of a polymorph (form II), which was much less soluble than the parent polymorph (form I) in the hydro-alcoholic medium of the formulation. Single-crystal XRD studies showed that both the hydrogen bonding capability and the packing density of

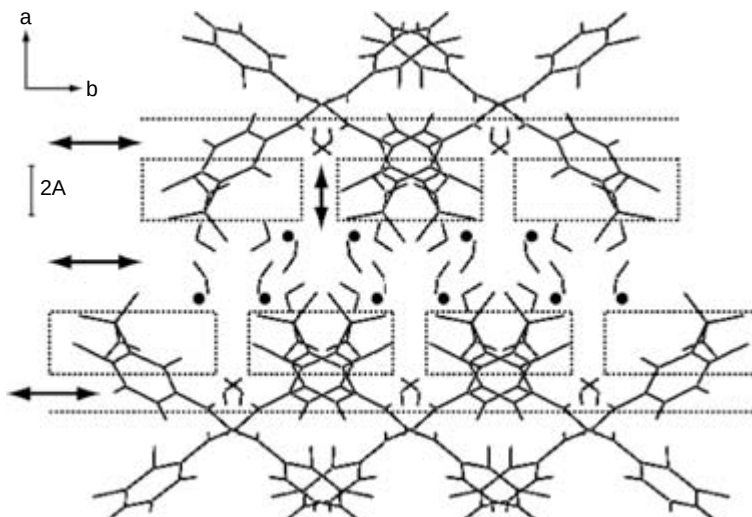


FIGURE 10 Top view along *c*-axis showing the crystal packing of form I of a developmental drug, the sodium salt of *N*-(3-(aminosulfonyl)-4-chloro-2-hydroxyphenyl)-*N'*-(2,3-dichlorophenyl) urea. Arrows indicate potential motion of water along the channels (*dashed lines*). Sodium atoms are represented by dark circles. *Source:* From Ref. (44).

form II was less than that of form I, which could be taken to suggest a greater stability of form I. However, the molecular conformation of ritonavir in form II was more favorable for crystal packing, and made this form the more stable polymorph.

It has been shown elsewhere that a thermodynamically stable polymorph may be characterized by the presence of unstable molecular conformations, such as the thermodynamically stable polymorph of oxybuprocaine hydrochloride, which was shown to have both stable and unstable molecular conformations in its crystalline forms (56).

X-Ray Powder Diffractometry

Although the solving of a crystal structure from an analysis of single-crystal diffraction provides the most fundamental structural understanding of polymorphic solids, the need for suitable single crystals and the degree of complexity associated with data analysis precludes this technique from being used on a routine basis for batch characterization. During routine synthesis, most drug substances are obtained as micro-crystalline powders, for which it is usually sufficient to establish only the physical form (usually polymorphic identity) of the solid, and to verify that the isolated compound is indeed of the desired structure. For these reasons, and in light of the experimental simplicity, the technique of XRD is the predominant tool for the study of polycrystalline materials (57–60), and is eminently suited for the routine characterization of polymorphs and solvates.

A properly prepared sample of a powdered solid will present a substantially random selection of all possible crystal faces and the diffraction patterns will therefore provide information regarding all possible spacings (atomic or molecular) in

the crystal lattice. To measure a powder pattern, a randomly oriented powdered sample is prepared in an effort to expose all the planes in the lattice. The scattering angle is determined by slowly rotating the sample and measuring the angle of diffracted X rays (typically using a scintillation detector) with respect to the angle of the incident beam. Alternatively, the angle between sample and source can be kept fixed, while moving the detector to determine the angles of the scattered radiation. Knowing the wavelength of the incident beam, the spacing between the planes (identified as the d -spacings) is calculated using Bragg's Law.

The XRD pattern will therefore consist of a series of peaks detected at characteristic scattering angles. These angles, and their relative intensities, can be correlated with the computed d -spacings to provide a full crystallographic characterization of the powdered sample. After indexing all the scattered lines, it is possible to derive unit cell dimensions from the powder pattern of the substance under analysis. For routine work, however, this latter analysis is not normally performed, and one typically compares the powder pattern of the analyte to that of reference materials to establish the polymorphic identity. Because every compound produces its own characteristic powder pattern owing to the unique crystal structure, powder XRD is clearly the most powerful and fundamental tool for polymorphic identity of an analyte.

The United States Pharmacopeia contains a general chapter on XRD (61), which sets the criterion that identity is established if the scattering angles in the powder patterns of the sample and reference standard agree to within the calibrated precision of the diffractometer. It is noted that it is generally sufficient that the scattering angles of the ten strongest reflections obtained for an analyte agree to within either ± 0.10 or $\pm 0.20^\circ 2\theta$, whichever is more appropriate for the diffractometer used. Older versions of the general test contained an additional criterion for relative intensities of the scattering peaks, but it has been noted that relative intensities may vary considerably from that of the reference standard, making it impossible to enforce a criterion based on the relative intensities of corresponding scattering peaks.

For identification purposes, it is usually convenient to identify the angles of the 10 most intense scattering peaks in a powder pattern, and to then list the accepted tolerance ranges of these based on the diffractometer used for the determinations. Useful tabulations of the XRD patterns of a number of compounds have been published by Koundourellis and coworkers, including 12 diuretics (62), 12 vasodilators (63), and 12 other commonly used drug substances (64).

The literature abounds with countless examples that illustrate how powder diffraction has been used to distinguish between polymorphs. It is safe to state that one could not publish the results of a phase characterization study without the inclusion of XRD data. Nowhere is this more crucial than in intellectual property controversies, where the different XRD patterns of polymorphs are used for patentability purposes. For example, two polymorphic forms of ibandronate sodium have been the subject of two United States patent applications (65), and although the XRD patterns of these are somewhat similar (Fig. 11), a sufficient number of differences in scattering peak angles exist so as to permit their identification. In the patent applications, some of the claims define the two polymorphs on the basis of five characteristic XRD scattering peaks, which enables one to tabulate the data and determine whether or not a given sample of ibandronate sodium is within the scope of these XRD claims.

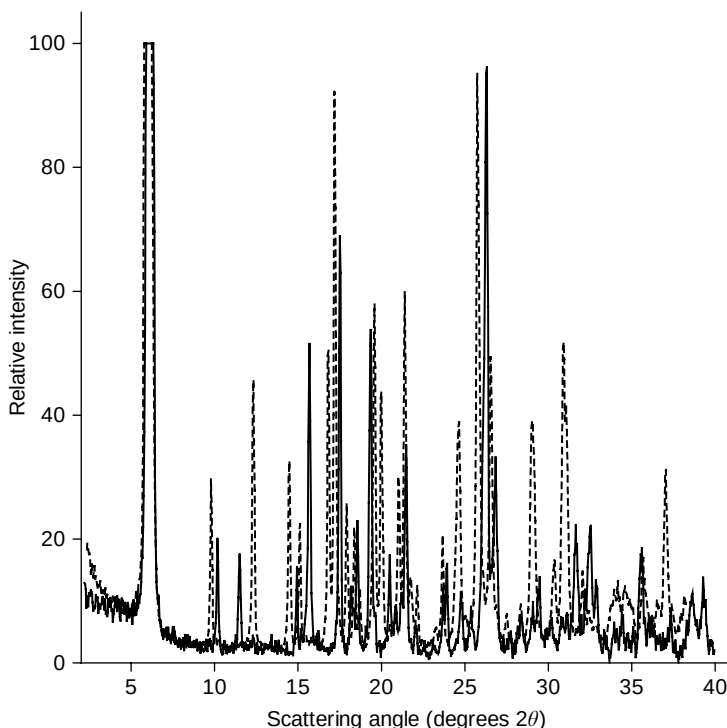


FIGURE 11 X-ray powder diffraction patterns of ibandronate sodium, Form A (*solid trace*) and Form B (*dashed trace*). The patterns have been adapted from data in United States patent applications 2006/0172975 and 2006/0172976 (65).

Once the characteristic XRD patterns of one or more analytes have been established, it is usually possible to develop methods for phase quantification. The methodology is based on the premise that each component will contribute to the overall scattering by an amount that is proportional to its weight fraction in a mixture, and that the powder pattern of each analyte contains one or more peaks whose scattering angle is unique to that analyte. Quantitative analysis typically requires the use of reference standards that contribute known scattering peaks at appropriate scattering intensities. This can be achieved through the use of internal standards, where one would mix reference materials such as elemental silicon or lithium fluoride into the sample matrix.

Although simple intensity correction techniques can be used to develop suitable quantitative XRD methods, the introduction of more sophisticated data acquisition and handling techniques can greatly improve the quality of the developed method. For instance, improvement of the powder pattern quality through use of the Rietveld method has been used to evaluate mixtures of two anhydrous polymorphs of carbamazepine and the dihydrate solvatomorph (66). The method of whole pattern analysis developed by Rietveld (67) has found widespread use in crystal structure refinement and in the quantitative analysis of complex mixtures.

Using this approach, the detection of analyte species was possible even when their concentration was less than 1% in the sample matrix. It was reported that good quantitation of analytes could be obtained in complex mixtures even without the requirement of calibration curves.

Well-characterized and stable (both physically and chemically) Standard Reference Materials are available from the National Institute of Standards and Technology (NIST) (68). These materials are used both to calibrate powder XRD equipment and to measure the instrument sensitivity. Silicon powder (SRM 640c) is typically used for the calibration of *d*-spacing or line position, and also can be used as an internal reference for quantitative applications. NIST SRM 674b is a set of four metal oxides (CeO_2 , Cr_2O_3 , TiO_2 , and ZnO) that are also used as internal standards in quantitative work.

The use of parallel beam optics as a means for determining the polymorphic composition in powder compacts has been evaluated (69). In this study, compressed mixtures of known polymorphic composition were analyzed in transmission mode, and the data processed using profile fitting software. The advantage of using transmission, rather than reflectance, was that the results were not sensitive to the geometrical details of the compact surfaces, and that spurious effects associated with preferential orientation were minimized.

The effects of preferred orientation in XRD analysis can be highly significant, and are most often encountered when working with systems characterized by plate-like or tabular crystal morphologies. A viable XRD sample is one that is free from the deleterious effects of preferential orientation, and any sample packing effect that serves to introduce a non-random pattern of crystal faces can strongly affect the observed intensities and any quantitative results. The problem has been investigated for the three needle-like polymorphs of mannitol, and it was found that through the use of small particles, the preferential orientation effects were held to a minimum, enabling quantification of the polymorphs around the 1% level (70).

The use of powder XRD has been investigated to determine whether one may use pattern indexing as another tool for polymorph screening studies (71). In this work, data were collected on six compounds using two diffractometers that employed transmission geometry, primary monochromatic radiation, and a position-sensitive detector. The data were found to exhibit good angular resolution, and therefore lattice parameters were easily obtained using the indexing program DICVOL-91. The extent of preferred orientation in each pattern was estimated using the DASH implementation of the March–Dollase function. It was concluded that the combination of experimental techniques used would be highly effective compared to just one technique that would require a large number of samples per day with the aim of obtaining the type of high-quality data necessary for pattern recognition and indexing.

Practical complications associated with preferential orientation effects have been discussed in detail (72). A number of sample-packing methods were considered (vacuum free-fall, front-faced packing vs. rear-faced packing, etc.), but the best reduction in preferential orientation was achieved by using materials having small particles that were produced by milling. Through the use of sieving and milling, excellent linearity in diffraction peak area as a function of analyte concentration was attained. The authors deduced a protocol for development of a quantitative XRD method consisting of (a) calculation of the mass attenuation coefficient of the drug substance, (b) selection of appropriate diffraction peaks for quantification,

(c) evaluation of the loading technique for adequate sample size, (d) determination of whether preferred orientation effects can be eliminated through control of the sample particle size, (e) determination of appropriate milling conditions to obtain reproducibility in peak areas, and (f) generation of calibration curves from physical mixtures.

Profile-fitting analysis has been combined with powder XRD to develop a quantitative method to determine the relative amounts of two prazosin hydrochloride polymorphs in their powder mixtures (73). Powder patterns of the pure α - and δ -polymorphs were initially obtained, and then patterns of calibration samples containing the α -form at concentrations ranging from 0.5% to 10% w/w in bulk δ -form. As is evident from Figure 12, the α -form exhibits a strong scattering peak at $27.5^\circ 2\theta$ where the δ -form exhibits little scattering, thus making this peak suitable for use in developing a quantitative method for phase composition. Using mathematical profile fitting, the background intensity level of the δ -form and the integrated intensity of peaks due to the α -form were determined, enabling a detection limit of 0.5%.

Powder XRD and diffuse reflectance infrared absorption spectroscopy analyses were used to develop two independent quantitative phase composition methods

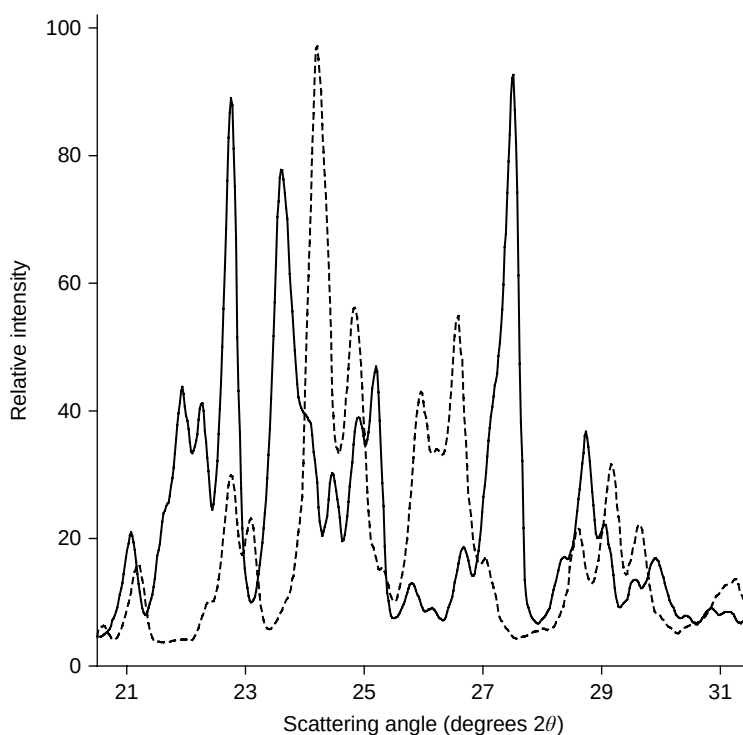


FIGURE 12 Expanded X-ray powder diffraction patterns of prazosin hydrochloride, α -form (solid trace) and δ -form (dashed trace), illustrating the peak at $27.5^\circ 2\theta$ that was used for quantitation purposes. The patterns have been adapted from data in Ref. (73).

for the determination of quantities of cefepime dihydrochloride dihydrate in samples of cefepime dihydrochloride monohydrate (74). For the XRD method, over a working concentration range 2.5% to 15% w/w, the limit of detection and quantification of the dihydrate in monohydrate were respectively 0.75% and 2.5% w/w. For the infrared method, a working range of 1.0% to 8.0% w/w was established with a minimum quantifiable level of 1.0% w/w and a limit of detection of 0.3% w/w dihydrate in monohydrate.

When reference samples of the pure amorphous and pure crystalline phases of a substance are available, calibration samples of known degrees of crystallinity can be prepared by mixing these reference samples. Establishment of a calibration curve (XRD response vs. degree of crystallinity) permits quantification of crystallinity in unknown samples. In one such study, a quantitative method was developed by beginning with commercially available crystalline digoxin and ball-milling of this substance to obtain various samples having various degrees of amorphous phase (75). Calibration mixtures were prepared as a variety of blends prepared from the 100% crystalline and 0% crystalline materials, and acceptable linearity and precision was obtained in the calibration curve of XRD intensity versus actual crystallinity.

In the absence of standard materials having known degree of crystallinity, one may determine the degree of crystallinity from the simple relation:

$$\text{DOC} = (A_c/A_T)(100) \quad (3)$$

where A_c is the integrated intensity attributable to the crystalline regions, and A_T is the total diffracted intensity. The evaluation of A_c and A_T is illustrated in Figure 13, where the reported XRD pattern of lenalidomide Form H (76) is shown along with its associated amorphous background. One would obtain A_c as the sum of the areas of the peaks above the dashed line, whereas A_T would be measured as the total area under the curve.

Determination of Crystal Structures from Powder Diffraction Data

As discussed above, a powder pattern will consist of a series of peaks having varying intensities, detected at various scattering angles. Through the indexing process, the d -spacings computed from the values of the scattering angles are assigned to diffraction from the crystal planes defined by their Miller (hkl) indices to provide a full crystallographic characterization of the powdered sample. After the indexing procedure is complete, it is possible to derive unit cell dimensions and other crystallographic information from a high-resolution powder pattern of a substance, eventually developing an approximate structural model that can be refined against experimental powder XRD data (structure refinement) (77–81). This procedure is best conducted on diffraction data collected using a high-flux synchrotron source (82).

Of the three crystal forms of telmisartan, the structure of solvated Form C was determined using single-crystal XRD and the structures of non-solvated forms A and B were determined from high-resolution powder XRD data using the method of simulated annealing for structure solution followed by Rietveld refinement (83). With 13 degrees of freedom (three translational, three orientational, and seven torsion angles), the structure solution was accomplished in approximately two hours of computer time, demonstrating that the crystal packing and the molecular conformation

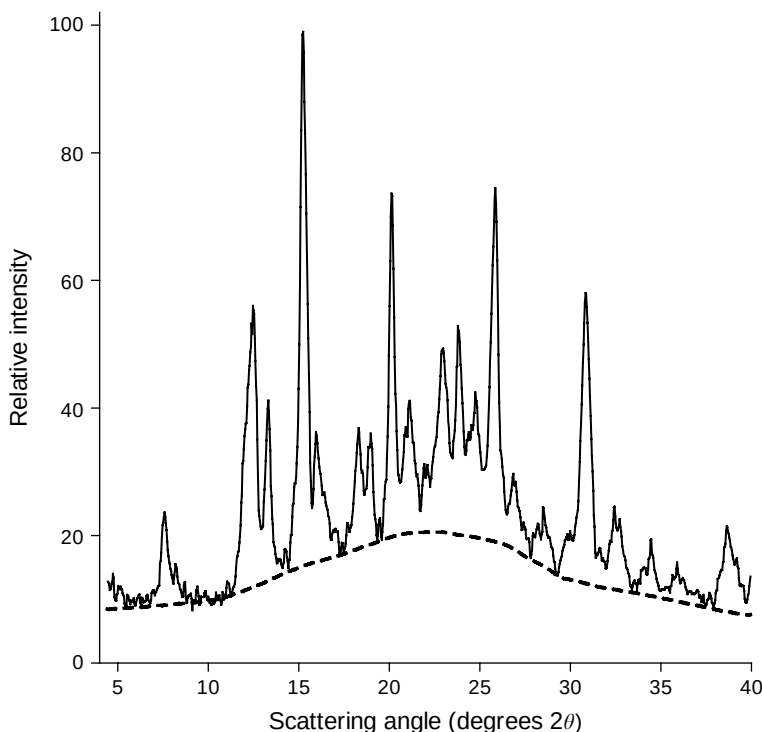


FIGURE 13 XRD pattern of lenalidomide form H (*solid trace*), and an estimation of the amorphous background (*dashed line*). The powder pattern of form H was adapted from data in Ref. (74).

of medium-sized pharmaceutical compounds could be solved quickly and routinely from high-resolution powder XRD data.

Other examples where structural properties have been derived from powder XRD data include enalapril maleate form II (84) and bupivacaine base (85).

Variable-Temperature X-Ray Diffractometry

XRD, performed on a hot stage, enables one to obtain powder patterns at elevated temperatures, and permits the deduction of structural assignments for thermally induced phase transformation reactions. By conducting VT-XRD studies, one can bring the system to conditions that had been indicated to be of interest during the conduct of thermal analysis studies. XRD systems equipped with hot-stage sample holders have been described, with this instrumentation being capable of obtaining powder patterns at temperatures of pharmaceutical interest (86,87). VT-XRD studies conducted under isothermal conditions can also be used for the calculation of activation energies of solid-state reactions.

VT-XRD is ideally suited to explain potentially complex solid-state transformations involving pharmaceutical hydrates. For example, Terakita et al. examined the dehydration behavior of calcium benzoate trihydrate, a system where the solid state transformations involved, in addition to crystalline and amorphous phases,

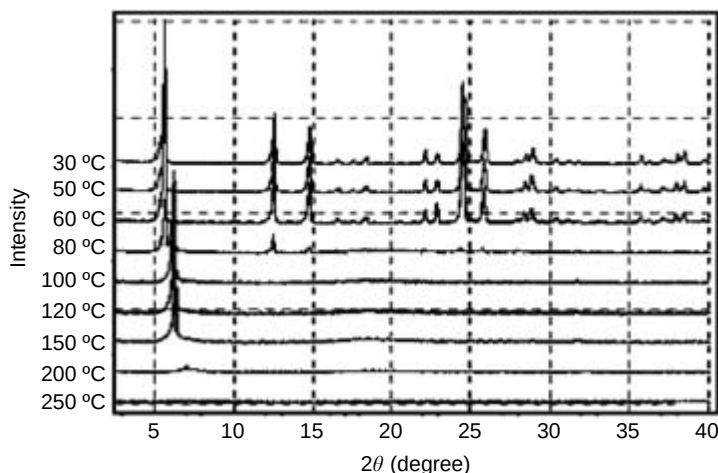


FIGURE 14 VT-XRD patterns obtained during the drying of calcium benzoate trihydrate, showing the gradual dehydration to a mesophase (100°C), which eventually culminated in the formation of an amorphous phase (250°C). *Source:* From Ref. (88).

a mesophase (88). As the temperature was increased, the trihydrate crystal form gradually formed a mesophase, as indicated by a sharp low-angle peak. As illustrated in Figure 14, further heating resulted in complete amorphization and the disappearance of all XRD peaks. A ground sample of the trihydrate was found to contain traces of calcium benzoate monohydrate, which was confirmed by VT-XRD to have formed by a recrystallization process. In this work, VT-XRD was shown to function both as an aid in the identification of crystalline phases, as well as in decoding the mechanism of their formation. Understanding such transformations and the knowledge of the conditions under which phase transitions occur, could aid in optimizing process parameters, such as the drying temperature of granules.

By virtue of their higher free energy compared to their more stable counterparts, metastable polymorphs of drug substances can significantly affect drug release from a dosage form. For example, theophylline monohydrate was observed by VT-XRD to dehydrate to the anhydrous form via an intermediate metastable anhydrate (89). The dissolution performance of tablets containing stable and metastable anhydrous theophylline was compared, where surprisingly the dissolution rate of the metastable form was found to be slower than that of the stable form. This phenomenon was shown to be due to the relatively rapid conversion of the metastable anhydrate form to the monohydrate form in the dissolution medium.

Isomorphic desolvates, formed by desolvation of solvates, represent the type of lattice structures that can be inherently unstable owing to their molecular packing. Consequently, such materials tend to be quite reactive and prone to instability, especially during their secondary processing into drug products (90). Miroshynk et al. used VT-XRD to investigate processing-induced phase transformations in erythromycin dihydrate (91). Dehydration resulted in shifts in the XRD peak positions, where some peaks shifted to higher angles owing to lattice expansion, whereas other peaks shifted to lower angular values as a result of lattice contraction.

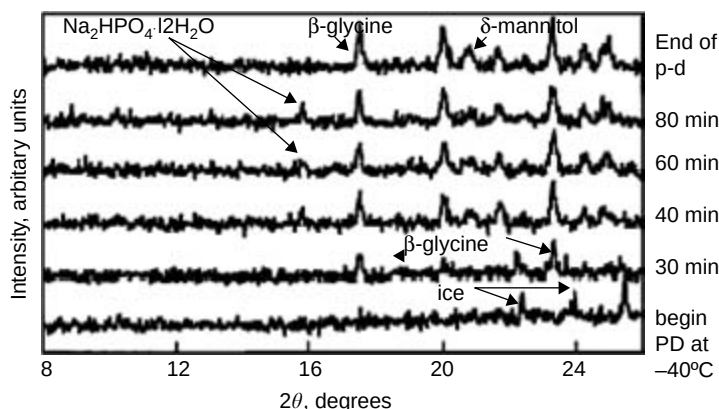


FIGURE 15 In situ VT-XRD patterns obtained during the primary drying (PD) of a frozen aqueous solution consisting of buffer salts, mannitol, and glycine. The solution was first cooled to -70°C at $2^{\circ}\text{C}/\text{min}$, which was then followed by heating to the primary drying temperature (-40°C) at $5^{\circ}\text{C}/\text{min}$. Primary drying was carried out for 4 hours. Source: From Ref. (92).

At higher temperatures, the XRD patterns indicated that the solids rearranged to form anhydrous erythromycin.

Although the typical use of VT-XRD is at elevated temperatures, the technique can also be used for work under sub-ambient conditions. For example, Pyne et al. characterized freeze-dried buffered aqueous systems of mannitol and glycine, and found that δ -mannitol and β -glycine crystallized during primary drying (92). By monitoring the intensities of the characteristic peaks of δ -mannitol and β -glycine both during primary and secondary drying, it was possible to determine the rate of crystallization of these solutes. Interestingly, one of the buffer salts, disodium hydrogen phosphate, crystallized as its dodecahydrate phase at the start of primary drying, but became dehydrated to an amorphous anhydrate at the end of the drying cycle (Fig. 15). It is important to note that conventional XRD of the final lyophile would not have revealed the crystallization of the buffer salt. The selective crystallization of disodium hydrogen phosphate only became evident by monitoring the phase transitions that took place *during* the freeze-drying process.

While attempting to simulate pharmaceutical processing steps, it is important to consider the effect of sample geometry on the phase transition kinetics. The relative quantities of stable and metastable anhydrous theophylline were found to be significantly different when theophylline monohydrate granules were dried in a microscale fluid bed dryer and in the sample stage of a powder X-ray diffractometer, with these differences being attributed primarily to differences in sample geometry (93). Although the VT-XRD sample bed was stationary, resulting in a slow drying rate, the sample in the fluid bed drier was dried rapidly due to fluidization.

XRD studies conducted at elevated temperatures can also be run under controlled water vapor pressures, using humidified nitrogen to control the vapor pressure. Han et al. proposed this technique to rapidly evaluate the physical stability of pharmaceutical hydrates, and studied the isothermal dehydration kinetics

of amoxicillin trihydrate at 68°C and at different water vapor pressures (94). The product they obtained was a poorly crystalline anhydrate. From these kinetic studies, the transition water vapor pressure of amoxicillin trihydrate was determined to be approximately 10.5 Torr at 68°C.

In addition to being extremely useful in the study of phase transformations, VT-XRD can also be used to obtain information on substance decomposition during a variable-temperature study. The commercially available form of aspartame is hemihydrate form II, which is known to transform into hemihydrate form I when milled, but a 2.5-hydrate species is also known (95,96). Through the use of VT-XRD, it was found that when either hydrate form was heated to 150°C, they dehydrated into the same anhydrous phase. Further heating of the anhydrate to 200°C resulted in a thermally induced cyclization, forming 3-(carboxymethyl)-6-benzyl-2,5-dioxopiperazine. The 2.5-hydrate was shown to dehydrate to hemihydrate form II when heated to 70°C, and this dehydration product was shown to undergo the same decomposition sequence as directly crystallized hemihydrate form II.

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