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Re.: Código 011-378-444
Expediente No. 10-132.023
Fase nacional de Solicitud
de Patente PCT
CELGENE CORPORATION

En relación con el expediente de la referencia y dando cumplimiento a la última providencia recaída sobre este asunto por medio del presente escrito me permito manifestarles lo siguiente:

1. Siguiendo precisas instrucciones modifíco el capítulo reivindicatorio de acuerdo con el ejemplar adjunto, debidamente revisado y corregido. Estas nuevas reivindicaciones están basadas en las antiguas reivindicaciones 4 a 9, y 25 a 32, y no incluyen reivindicaciones sobre métodos de tratamiento, y corresponde al grupo inventivo 2 mencionado en el oficio.
2. Con respecto a las reivindicaciones que contienen datos XRPD, la sociedad solicitante manifiesta que la información que se provee en estas reivindicaciones es suficiente para que una persona versada en la materia identifique las formas cristalinas reclamadas del compuesto. Las reivindicaciones 1-3 reclaman picos específicos XRPD en grados 2θ, y la reivindicación 4 reclama el espectro específico XRPD mostrado en la figura 5 de la descripción. Se adjunta copia de las páginas 1-42 de la edición del año 2010 de la publicación Pharmaceutical Sciences Encyclopedia of Drug Discovery, Development and Manufacturing, la cual enseña que XRPD es aceptado como el "estándar de oro" de las técnicas de identificación para la identificación de polimorfos. Una persona versada en la materia es capaz de identificar la Forma B del compuesto de fórmula (I) solamente basada en la información XRPD proporcionada en cada una de las reivindicaciones 1 a 4.

3. El Examinador también sostiene que las reivindicaciones deben incluir las intensidades correspondientes del espectro XRPD. En la página 6 del documento enunciado en el punto anterior se enseña que la intensidad pico es proporcional al grado de orientación preferida, que varía de experimento en experimento. Por lo tanto, el agregar un pico de intensidad a las actuales reivindicaciones modificadas no es necesario para caracterizar adecuadamente la Forma B del compuesto de fórmula (I).
4. Similarmente, la reclamación de picos XRPD en grado 2θ en las reivindicaciones 1 a 3 es suficiente para una persona versada en el arte para caracterizar la forma cristalina B del compuesto de fórmula (I). No hay necesidad de identificar la forma de cristal a través de otra técnica en estas reivindicaciones, tal y como se aprecia del documento adjunto mencionado.
5. Al contrario de lo que el Examinador considera, mi representada cree que la descripción presentada describe de manera suficiente y clara un proceso para la preparación de la Forma B del compuesto de la fórmula (I). En la página 27 de la solicitud internacional se describen dos métodos para la preparación de la forma cristalina B de una muestra del compuesto de fórmula (I). El primer método es realizado por cristalización del compuesto de fórmula (I) de un sistema solvente etanol:agua (1:1) por evaporación a aproximadamente 25°C (párrafo 00120). El segundo método es realizado suspendiendo el compuesto de fórmula (I) en etanol:acetona (1:1) a aproximadamente 25°C por más o menos 2 días. Basado en estos ejemplos, el forma cristalina B del compuesto de fórmula (I) puede ser preparado utilizando técnicas experimentales comúnmente usadas por una persona versada en el arte.
6. Ahora bien, el Examinador argumenta que la presente solicitud tiene falta de novedad y nivel inventivo debido a la previa existencia de D1 WO03080049. Mi representada argumenta que el proceso revelado en D1 para la preparación del compuesto de fórmula (I) no es el mismo que el proceso para preparación de las formas cristalinas específicas de la presente solicitud. Más aun, D1 no revela una forma cristalina B enantioméricamente pura de las presentes reivindicaciones. Contario a lo sostenido por el examinador esa solicitud de patente revela diferentes métodos para la

- preparación del compuesto de formula (I) al compararse con los métodos descritos en D1.
7. En la solicitud en estudio, la Forma B del compuesto de fórmula (I) de las reivindicaciones modificadas se prepara a partir de 2-[1-(3-etoxi-4-metoxifenil)-2-metilsulfoniletil]-4-acetamidoisoindolin-1,3-diona enantioméricamente pura mediante la cristalización de un sistema de solvente etanol: agua (1:1) por evaporación a una temperatura de cerca de 25°C. Ver el párrafo [00120]. Como alternativa, la Forma B se prepara haciendo una lechada del compuesto de fórmula (I) etanol: acetona (1:1) a una temperatura de cerca de 25°C durante cerca de 2 días. *Id.* Estos procedimientos específicos no se describen en el documento D1 En efecto, D1 no especifica las condiciones de recrystalización aparte del sistema de solventes. En particular, D1 no se refiere a las condiciones de lechada de dos días descritas en la presente solicitud para obtener la Forma B.
8. Una comparación estrecha de las diversas condiciones descritas en D1 y la especificación en estudio revela que la forma sólida obtenida en el proceso de recrystalización de D1 puede ser una forma sólida diferente de la Forma B, como la Forma A. Les rogamos considerar proporcionar la siguiente tabla con su respuesta con el fin de demostrar las diferencias descritas entre D1 y la presente solicitud:

	D1: Producto sólido	Solicitud en estudio: Forma A	Solicitud en estudio: Forma B
	Página 29, l. 10-12	Página 25, [00113]	Página 27, [00120]
Sistema de solventes	Acetona: etanol 1: 2	Acetona, etanol y mezclas de los mismos	Acetona: etanol 1: 1
Condiciones ejemplares	No se especifica	Cristalización por enfriamiento rápido	Formación de lechada a 25°C durante 2 días

Como muestra la tabla, el sistema de solventes que se usa en D1 (acetona: etanol 1:2) difiere del sistema de solventes ejemplar de la presente solicitud por la obtención de la Forma B (acetona: etanol 1:1). No obstante, el sistema de solventes que se usa en D1 (acetona: etanol 1:2) está incluido dentro de los sistemas generales de solventes descritos para obtener la Forma A en lugar de la Forma B. Dado que el proceso mencionado en D1 no está suficientemente definido para proporcionar una revelación directa e inequívoca de alguna forma sólida específica, sin mencionar la Forma B específica de las reivindicaciones en estudio, D1 no puede prever las reivindicaciones en estudio de la Forma B. Por lo menos con base en las anteriores razones, les pedimos que le soliciten al Examinador que retire la objeción de novedad.

9. Con respecto al nivel inventivo, la capacidad de un compuesto químico de existir en una o más formas cristalinas estables específicas es impredecible. Por ejemplo, el simple conocimiento de la estructura química del compuesto de fórmula (I) no sugiere que este compuesto se pueda precipitar como material cristalino, ni mucho menos la forma cristalina B específica de las reivindicaciones en estudio. Aun cuando se sabe que un compuesto químico forma un sólido cristalino, suele ser impredecible si el compuesto va a formar más de una forma sólida, y si el sólido será cristalino o amorfo. Por ejemplo, un artículo del año 2006 del Materials Research Bulletin explica que "en general no es posible predecir de forma computacional el número de polimorfos observables incluso de las moléculas más sencillas". Ver Jones, W. et al., Mater. Res. Bull. (2006), 312375-79, en 875 (se anexa una copia). Como resultado del alto grado de impredecibilidad en este campo, los investigadores tienen que realizar experimentación detallada para verificar y entender la formación de las formas sólidas que contienen un compuesto farmacéutico en particular. Además, la solicitud en estudio enseña que la Forma B del compuesto de fórmula (I) tiene propiedades físicas mejoradas como ingrediente farmacéutico activo, en comparación con otras formas sólidas del compuesto. Por ejemplo, los datos de la especificación en estudio demuestran que las formas cristalinas del compuesto de fórmula (I) pueden fácilmente interconvertirse en condiciones particulares. Ver las Tablas 11 y 12 de la descripción.

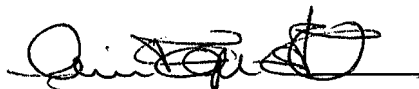
D1 no enseña ni sugiere nada con respecto a las condiciones particulares que se necesitan para preparar de forma reproducible alguna forma cristalina específica, mucho menos la Forma B de las reivindicaciones en estudio, y D1 tampoco proporciona ninguna información sobre la estabilidad de dichas formas cristalinas.

10. Se ha demostrado que la Forma B es estable en diversas condiciones como humedad, temperatura y presión elevadas. Ver el párrafo [00124] y la Tabla 11. Se encontró que la Forma B es particularmente estable en comparación con otras formas cristalinas del compuesto. Ver las Tablas 11 y 12.
11. En resumen, la forma cristalina B específicamente reivindicada no podría haber sido prevista por la persona medianamente versada en la materia técnica correspondiente a causa del alto grado de impredecibilidad del campo. Además, la inesperada estabilidad de la Forma B en comparación con otras formas cristalinas del compuesto de fórmula (I) aquí reveladas representa un nivel inventivo adicional. Ver las Tablas 11 y 12 de la especificación.

Adjunto por lo tanto el comprobante de pago por concepto de exceso de reivindicaciones en el capítulo modificado por valor de \$ 450.000.00, recibo No. 28,472

Renuncio al término faltante de traslado, y les solicito otorgar el privilegio de patente de invención aquí reclamado.

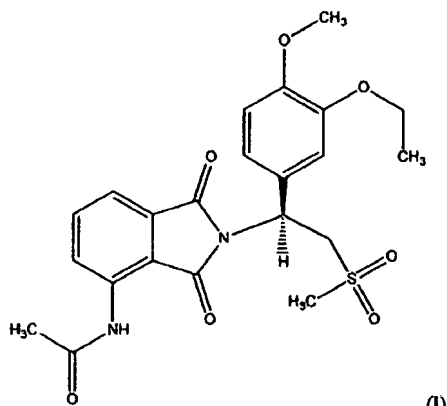
De Ustedes Atentamente,



LUIS FELIPE CASTILLO GIBSON
T.P. No. 68.995 del C.S.J.

Reivindicaciones

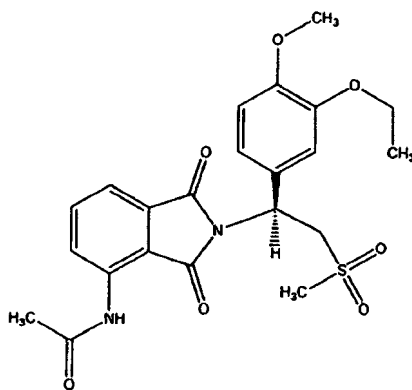
1. Una forma sólida cristalina enantioméricamente pura del compuesto de fórmula (I),



(I)

la cual es la forma cristalina B sustancialmente pura, y la cual tiene un patrón de difracción de rayos X del polvo que comprende picos a los 10,1, 13,5, 20,7 y 26,9 grados 2 θ .

2. La forma cristalina de la reivindicación 1, la cual tiene un patrón de difracción de rayos X del polvo que además comprende picos en los 12,4, 15,7, 18,1, y 24,7 grados 2 θ .
3. La forma cristalina de la reivindicación 2, la cual tiene un patrón de difracción de rayos X del polvo que además comprende picos en los 16,3, 22,5, 26,2, y 29,1 grados 2 θ .
4. La forma cristalina de la reivindicación 1, la cual tiene un patrón de difracción de rayos X del polvo que se corresponde con el patrón ilustrado en la FIG. 5.
5. Una forma sólida cristalina enantioméricamente pura del compuesto de fórmula (I):



(I)

la cual es la forma cristalina B sustancialmente pura, y la cual tiene una gráfica de calorimetría diferencial de barrido que comprende un evento endotérmico con una temperatura de inicio de 154°C.

6. La forma cristalina de la reivindicación 5, la cual tiene una gráfica de calorimetría diferencial de barrido que se corresponde con la gráfica ilustrada en la FIG. 6.
7. La forma cristalina de la reivindicación 5, la cual tiene una gráfica de análisis gravimétrico térmico que comprende una pérdida de masa de menos de 1% cuando se calienta de 25°C a 140°C.
8. La forma cristalina de la reivindicación 7, que se caracteriza porque la pérdida de masa es de 0,25%.

- 7
9. La forma cristalina de la reivindicación 1, la cual tiene una gráfica de análisis gravimétrico térmico que se corresponde con la gráfica ilustrada en la FIG. 7.
 10. La forma cristalina de la reivindicación 1, la cual exhibe un incremento de masa de menos de 1% cuando se somete a un aumento de la humedad relativa de 0% a 95% de humedad relativa.
 11. La forma cristalina de la reivindicación 10, que se caracteriza porque el incremento de masa es de 0,6%.
 12. La forma cristalina de la reivindicación 1, la cual tiene una gráfica de la isoterma de la sorción de humedad que se corresponde con la gráfica ilustrada en la FIG. 8.
 13. La forma cristalina de la reivindicación 1, la cual es estable luego de la exposición a 40°C y 75% de humedad relativa durante 4 semanas.
 14. Una composición farmacéutica apropiada para administración oral, que comprende de 10 mg a 200 mg de la forma cristalina de cualquiera de las reivindicaciones 1-13.
 15. La composición farmacéutica de la reivindicación 14, que se caracteriza porque la composición farmacéutica comprende de 10 mg a 100 mg de la forma cristalina.
 16. La composición farmacéutica de la reivindicación 14, que se caracteriza porque la composición farmacéutica se encuentra en forma de cápsula.
 17. La composición farmacéutica de la reivindicación 16, que se caracteriza porque la cápsula contiene 10 mg del compuesto.
 18. La composición farmacéutica de la reivindicación 16, que se caracteriza porque la cápsula contiene 20 mg del compuesto.
 19. La composición farmacéutica de la reivindicación 16, que se caracteriza porque la cápsula contiene 25 mg del compuesto.
 20. La composición farmacéutica de la reivindicación 16, que se caracteriza porque la cápsula contiene 50 mg del compuesto.
 21. La composición farmacéutica de la reivindicación 14, que se caracteriza porque la composición farmacéutica se encuentra en forma de tableta.
 22. La composición farmacéutica de la reivindicación 21, que se caracteriza porque la tableta contiene 10 mg del compuesto.
 23. La composición farmacéutica de la reivindicación 21, que se caracteriza porque la tableta contiene 20 mg del compuesto.
 24. La composición farmacéutica de la reivindicación 21, que se caracteriza porque la tableta contiene 25 mg del compuesto.
 25. La composición farmacéutica de la reivindicación 21, que se caracteriza porque la tableta contiene 50 mg del compuesto.

Uses of X-Ray Powder Diffraction In the Pharmaceutical Industry

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1 INTRODUCTION

Among the many experimental techniques available for the identification of solid forms, including polymorphs, solvates, salts, cocrystals and amorphous forms, X-ray powder diffraction (XRPD) stands out as a generally accepted "gold standard." While this does not mean that XRPD should be used to the exclusion of other experimental techniques when studying solid forms, X-ray diffraction (XRD) has applications throughout the drug development and manufacturing process, ranging from discovery studies to lot release. The utility of X-ray diffraction becomes evident when one considers the direct relationship between the measured X-ray diffraction pattern and the structural order and/or disorder of the solid. XRPD provides information about the structure of the underlying material, whether it exhibits long-range order as in crystalline materials, or short-range order as in glassy or amorphous materials. This information is unique to each structure—whether crystalline or amorphous—and encoded in the uniqueness of the XRPD pattern collected on a well-prepared sample of the material being analyzed.

One must draw a distinction between crystalline materials, which give rise to XRPD patterns with numerous well-defined sharp diffraction peaks, and glassy or amorphous

TABLE 1 Types of Solid Forms Described by the Wunderlich (1) Classification System

Solid Form	Translation	Orientation	Conformation
Crystal	Long range	Long range	Long range
Condis crystal (glass)	Long range	Long range	Short range
Plastic crystal (glass)	Long range	Short range	Short range
Liquid crystal (glass)	Short range	Long range	Short range
Amorphous (glass)	Short range	Short range	Short range

materials whose XRPD patterns contain typically three or less broad maxima (X-ray amorphous halos). In practice, using XRPD, one can usually measure a sequence of progressively more disordered crystalline materials that ultimately result in glass. A classification system has been proposed by Wunderlich (1), Table 1, to describe the type of structural order and molecular packing present in molecular organic solid forms using three order parameter classes: translation, orientation, and conformation.

Solid forms of a given molecule exhibiting long-range crystalline order (e.g., polymorphs, solvates, co-crystals, and salts) can be identified and characterized using XRPD by their unique combination of order parameters. Amorphous solid forms do not exhibit any long-range order but are identifiable and characterized by their unique local molecular order, apparent in the X-ray amorphous diffraction pattern (2).

Knowing that X-ray powder diffraction is sensitive to structural order, some of its typical applications in the analysis of solid-state properties of a drug substance or product include:

- Identification of existing forms of the active pharmaceutical ingredient (API).
- Characterization of the type of order present in the API (crystalline and/or amorphous).
- Determination of physical and chemical stability.
- Identification of the solid form of the API in the drug product.
- Identification of excipients present in a drug product.
- Monitoring for solid form conversion upon manufacturing.
- Detection of impurities in a drug product.
- Quantitative analysis of a drug product.

Where appropriate data are available, XRPD analysis can determine the solid-form structure and crystal-packing relationship among individual molecules in the solid. This information is essential to the understanding of solid-state chemistry of drugs and important from the regulatory perspective.

2 X-RAY DIFFRACTION THEORY

When dealing with organic samples, a common simplification called the first Born approximation (3,4) is useful in explaining the X-ray diffraction process. Solid forms

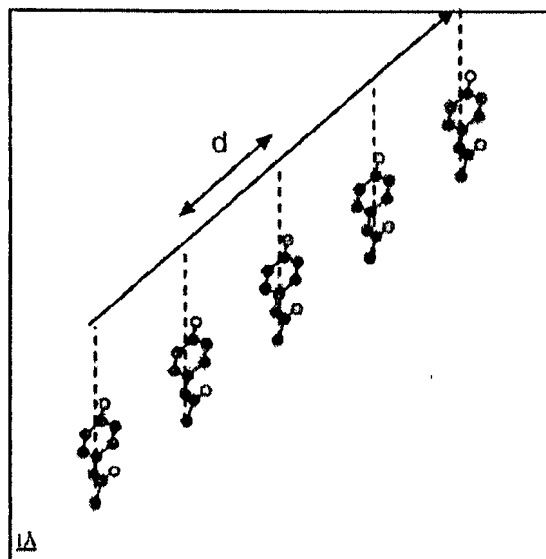


FIGURE 1 A simple periodic array of molecules with a single orientation and conformation and constant spacing, d .

of organic materials are expected to interact weakly with incident X-rays (generally true provided the crystallite size is not too large). This means that the amplitude of doubly and multiply scattered radiation will be very small and negligible when compared to the singly scattered radiation (4). In the presence of crystal defects, grain boundaries or disordered systems (i.e., typical laboratory samples and not large, perfect single crystals), the multiply scattered radiation becomes even less significant.

Respecting the limits of these assumptions, we can model the diffraction process as a Fourier transform of the electron density within the sample. Figure 1 shows a simple example containing a periodic array of molecules separated by a constant spacing, d , and with a single orientation and conformation. While each atom is considered a point source of scattering (5), the molecules themselves can be reduced to point sources of scattering under the assumption that the electron density distribution of a collection of atoms is the sum of the electron density distributions attributed to individual centered atoms (4). Note that atoms in a molecule are not necessarily the same as isolated, free atoms, though they are often approximated as such. The latter approximation allows us to substitute values for the Fourier transform of electron density of each individual atom using the tables of atomic scattering factors (6).

During the diffraction process, the ordered arrangement of point scattering centers in real space produces a set of diffraction events in reciprocal space corresponding to sharp peaks. A spacing of d between the point centers (molecules) in real space will correspond to a peak spacing of $2\pi/d$ in reciprocal space (also called Q -space). This simple Fourier identity, illustrated in Fig. 2, will apply to any molecular-translational order existing within a solid form (4).

Therefore, diffracted peak positions can be expressed in terms of d -space, Q -space or, most commonly, 2θ , the angle between diffracted and undeviated X-ray waves (5).

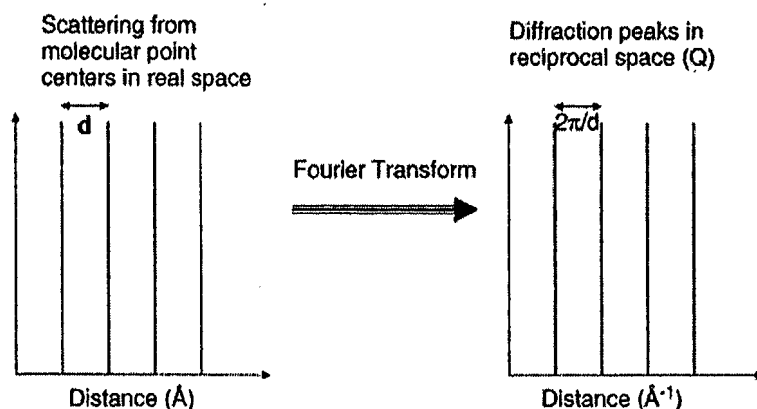


FIGURE 2 A Fourier transform relates a periodic array of molecular point scattering centers in real space to a family of diffraction peaks in reciprocal space.

Bragg's law can be used to relate the X-ray half-scattering angle θ to the aforementioned spacing parameter, d , as seen in Eq. 1.

$$n\lambda = 2d\sin\theta \quad (1)$$

The parameter λ is the wavelength of the incident X-ray radiation, with a typical value of approximately 1.54 Å for a Cu X-ray radiation source. n is an integer and can best be understood through the explanation of why Bragg's law holds. Consider a case of many parallel scattering planes—each of which is a collection of previously introduced point-scattering centers—reflecting incoming X-ray radiation of wavelength λ at the angle θ , as seen in Fig. 3 (5). The two diffracted waves in Fig. 3 will interfere with each other either constructively (adding together to produce stronger peaks) or destructively (subtracting from each other to some extent), depending on whether they are in phase or out of phase, respectively (4). Since the wave diffracted by the bottom plane in Fig. 3 travels a greater distance (greater by exactly $a + b$) than the wave diffracted by the top plane, that extra distance must be equal to an integer multiple of λ for points to remain in phase and total constructive reinforcement to

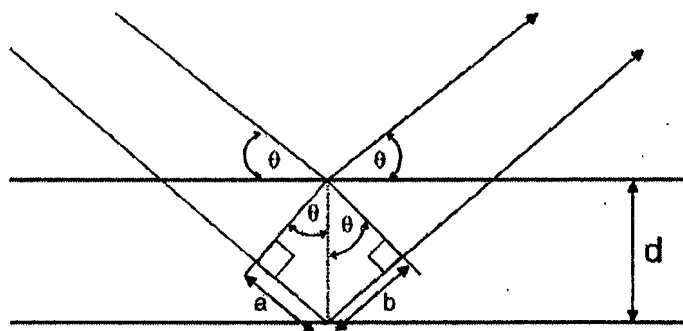


FIGURE 3 Bragg's law for parallel planes.

occur between the scattering from these planes (Eq. 2).

$$n\lambda = a + b \quad (2)$$

a and b can be expanded using the law of sines as $\sin\theta = a/d = b/d$. Simple substitution then transforms Eq. 2 into Bragg's law (1). The integer n refers to the order of the diffraction. For additional readings on X-ray diffraction theory, see for example References (4–8).

3 X-RAY DIFFRACTION EXPERIMENTAL PROCEDURES

XRPD patterns display diffracted intensity as a function of the experimental parameter 2θ (angle between diffracted and undeviated X-ray waves). Intensity is typically expressed in counts or counts per second while peaks are listed as positions in $^\circ 2\theta$ or d -spacings (usually measured in Å or nm).

3.1 Crystalline Materials

For materials exhibiting long-range (crystalline) order, XRPD patterns will contain sharp peaks, Fig. 4, whose shape and width will depend on the type of instrument on which the data were collected. Measurement ranges for crystalline materials depend on the type of study. For example, in a common application of XRPD—small-molecule polymorphism study— $1\text{--}40^\circ 2\theta$ is a typical measurement range used, since

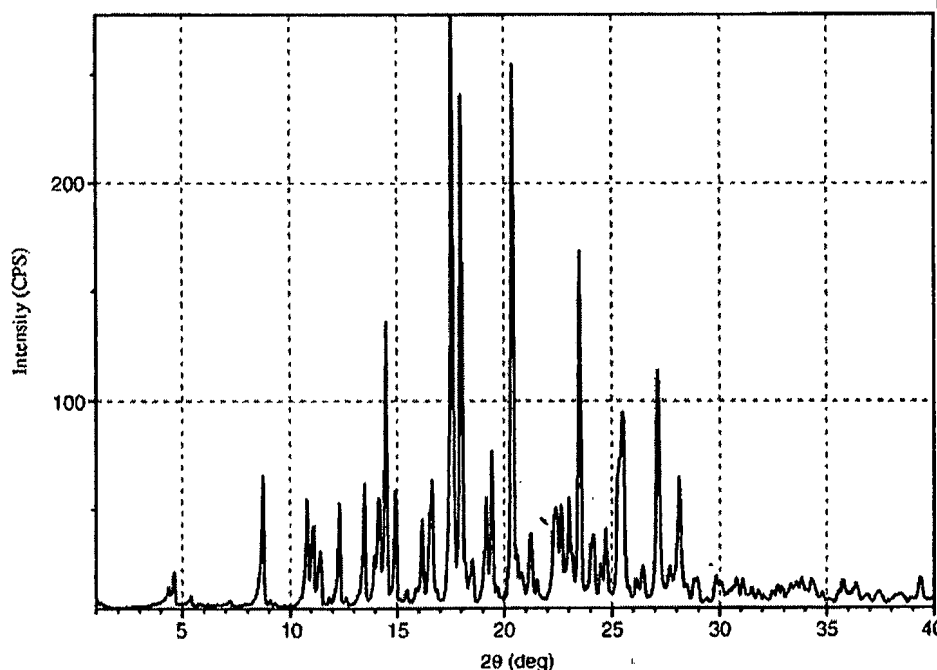


FIGURE 4 XRPD pattern of crystalline itraconazole.

peaks above approximately $30^\circ 2\theta$ become too numerous and overlapped to be useful in differentiating between polymorphs. When working with large molecules (e.g., biologicals), it is advantageous to measure to as low an angle as allowed by the geometry of the instrument (approximately $0.5^\circ 2\theta$ is achievable in typical laboratory settings with modern instruments, sub $0.5^\circ 2\theta$ using a synchrotron or with a dedicated small-angle scattering instrument). Certain computational methods may require measurements to significantly higher 2θ angles, up to $100^\circ 2\theta$.

Collection time varies per application but, for polymorphism studies, good patterns of crystalline material on modern instruments can be obtained in 2–10 min. In high throughput configurations for well plates, collection times of less than a minute per pattern can readily be achieved. Depending on the instrument geometry and application, 2–20 mg of crystalline sample may be required, and the sample can be reused afterwards as XRPD is typically non-destructive.

XRPD suffers from two common sample-related effects that can play a significant role when characterizing or identifying crystalline material. Ideal XRPD samples have large numbers of randomly oriented crystallites. The reproducibility of an XRPD pattern is dependent on particle-orientation statistics, while preferred orientation limits the degree to which a pattern accurately represents the structure, as opposed to a particular sample preparation. For those reasons, one must assess the particle statistics and degree of preferred orientation before one can be confident the peak identifications made from a particular pattern are representative of the material.

The effect of non-random (preferred) orientation of crystallites in a sample is to increase the relative intensities of some peaks and decrease the relative intensities of others. The variation in peak intensity is proportional to the degree of preferred orientation. In extreme cases, it can cause peaks to disappear completely while exaggerating otherwise faint peaks. Even in mild cases, a different set of relative intensities would result from diffractometers with different sample geometries.

Poor particle statistics are displayed by samples where a relatively small number of crystallites contribute to the integrated XRPD patterns. Since the small population of large crystallites cannot represent all possible orientations, the measured relative intensities are not reproducible. Irreproducible peak intensities lead to the expectation that the pattern may differ dramatically if the same experiment was repeated on a different sample of the same material even using the same diffractometer.

Both preferred orientation and poor particle statistics can be reduced to some extent in laboratory samples by spinning the sample in a holder (9, 10). Diffractometers allow the sample to be spun around one or two axes. In addition, a simple way of assessing the degree of preferred orientation or particle statistics in a sample is to analyze it on two different diffractometers with different spinning geometries. A summary of methods to minimize preferred orientation can be found in References (11–14). Bish (5) includes a detailed discussion on sample preparation and related problems.

3.2 X-Ray Amorphous Materials

In the case of X-ray amorphous (disordered, glassy or amorphous) materials, there will be no sharp peaks observed in the XRPD pattern, only broad halos (Fig. 5).

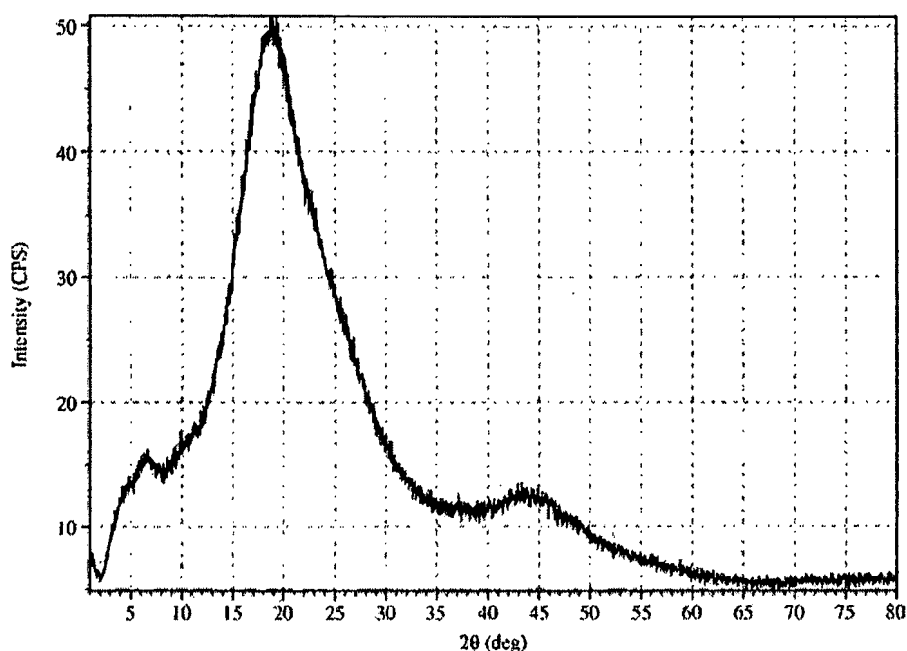


FIGURE 5 XRPD pattern of amorphous itraconazole.

Nevertheless, as we shall see later on, it is possible to extract structural information from these types of patterns using computational methods. Many of the computational methods used to analyze disordered materials require that the data are collected over a broad range, typically from 1 to $100^\circ 2\theta$. Furthermore, because the signal-to-noise ratio in X-ray amorphous patterns is typically poor, longer collection times are often used. 30–60 min is not uncommon for a single pattern. Finally, a greater amount (50–100 mg) of amorphous sample is typically warranted to obtain a good XRPD pattern.

XRPD patterns of both crystalline and X-ray amorphous materials contain some experimental artifacts, for example, instrument-background functions, sample-holder fingerprints, incoherent (Compton) scattering, polarization and Lorentz effects, and air scatter (5). The relatively low signal generated from X-ray amorphous samples means those artifacts will represent a much greater portion of the overall diffracted intensity. Therefore, computational methods used to analyze X-ray amorphous materials (15–17) are very sensitive to experimental artifacts and care must be taken to minimize their presence. Instrument intensity-correction functions can be determined using known standards and computationally modeled thereafter. Sample holders that produce significant X-ray scattering of their own in the relevant regions (e.g., glass capillaries) should be avoided when working with amorphous materials. Instead, samples can be sandwiched between thin polymer films with low X-ray background (e.g., Etnom[®]). Given the chemical formula of the material, Compton scattering can be calculated from atom-scattering tables (6) and subtracted from the overall signal. Air scatter can be largely eliminated using custom-built enclosures for a helium atmosphere.

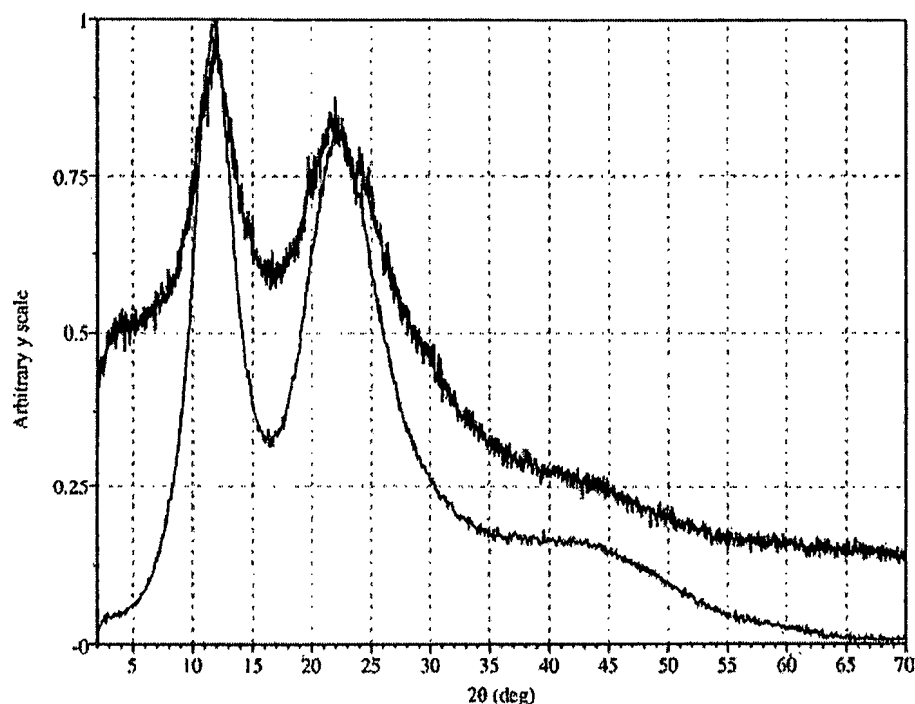


FIGURE 6 The effects of experimental artifacts on amorphous X-ray patterns. Both patterns were collected from the same sample of amorphous nifedipine. The top pattern was collected from approximately 10 mg of material packed in a glass capillary under ambient conditions. The collection time was 5 min. The bottom pattern was collected from approximately 100 mg of material sandwiched between two thin sheets of Etnom[®] film in a He-purged enclosure. The collection time was 1 h.

Figure 6 shows an X-ray amorphous pattern of nifedipine collected on a standard instrument with no special considerations and the sample packed in a glass capillary, compared to the same sample collected on a specially configured instrument using a low background sample holder, He atmosphere, and longer collection time. Note the patterns in this figure have not been offset but normalized to top intensity. As seen in the bottom pattern of Fig. 6, the low angle air scatter contribution is largely eliminated through the use of He. Furthermore, the broad glass scattering around $25^{\circ}2\theta$ which causes a shift in the position of the second amorphous halo in the top pattern is not evident in the bottom pattern. Typically, in screening applications, no more than a few milligrams of material are available, resulting in patterns where the glass/air scatter signal overwhelms the signal from the material itself and little structure is evident.

Certain types of analyses involving crystalline materials (e.g., quantitative studies or indexing) may also benefit from careful experimental setups but the most common applications (i.e., polymorph detection or identification (18)) generally do not require the specialized setups or longer collection times used for amorphous materials.

3.3 Instrumentation

Laboratory X-ray diffractometers typically consist of an X-ray source, sample chamber and detector. The most commonly used X-ray source in laboratory experiments with organics is Cu, though instruments typically allow different sources to be used with some configuration. Slits and optics are used to focus the incident and diffracted radiation on the sample and detector, respectively. Specimens usually can be rotated to alleviate some of the intensity artifacts discussed earlier. Detectors can be point, line or area, with the latter offering advantages in both speed of acquisition and ability to assess the particle statistics and preferred orientation in a sample through examination of Debye rings (19). Synchrotron sources are sometimes used for specialized measurements to collect high quality data. Detailed descriptions of X-ray instrumentation can be found, for example in Reference (5).

The alignment of a diffractometer is maintained through the use of calibration standards, such as silicon, to verify the position calibration (5). Regular calibrations are accepted industry practice (cGMP), with the frequency of calibration ranging from every X number of samples to set time periods (e.g., daily), depending on how much data one is willing to risk invalidating due to a failed calibration. When a silicon standard is used, the Si 111 peak position is typically verified using a short scan in the appropriate region (around $28.4^\circ 2\theta$). Other standards may be used, for example, to verify low angle alignment.

Diffractometers can typically be operated in either reflection or transmission configurations. Reflection is by far the more common and also referred to as Bragg-Brentano geometry (Fig. 7). In reflection measurements, incoming X-rays are "reflected" off the sample surface and focused by the instrument optics onto the detector. Errors due to sample transparency to X-rays are common for organic samples (5,14). The X-rays penetrate many atomic layers below the surface meaning the average diffracting surface lies somewhat beneath the surface of the sample. This type of error can lead to peak position errors in measured patterns of as much as a tenth of a degree. Therefore, low absorbing samples are often prepared as thin films using

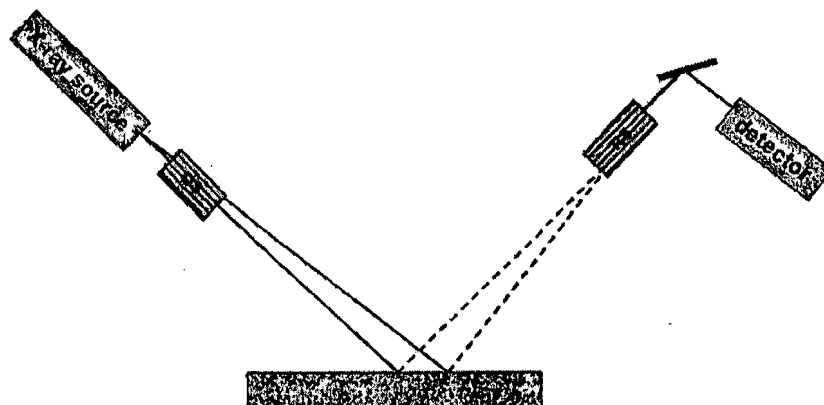


FIGURE 7 Bragg-Brentano diffractometer geometry.

a low background holder, to reduce the penetration effect. Displacement errors arise from the difficulty of preparing samples such that the surface of the sample is level with the surface of the holder (where the instrument is focused). Sample-displacement errors can result in significant, systematic shifts in measured peak positions, on the order of several tenths of a degree in particularly bad cases. Experience in preparing samples such that they are level with the surface of the holder is the only solution to this problem, although computational methods can be used to shift the pattern and correct peak positions, where the error is recognized. A further limitation of reflection measurements is the inability to measure to very low angles—typically $2.5^\circ 2\theta$ is a practical limit—making this type of measurement less useful for the analysis of large molecules which are expected to have peaks in the 0 – $2.5^\circ 2\theta$ range.

Transmission mode analysis overcomes many of the limitations of reflection mode when carefully configured. In transmission measurements, the incident X-rays pass through the sample and are diffracted not only on the surface but throughout the sample. This type of analysis is possible for organics due to their relative transparency to X-rays. The sample no longer needs to be level with the holder but thickness of the sample is important and can cause peak displacement errors (when the sample is too thick). Transmission configurations generally allow lower angle measurements than reflection, making them useful for large molecules. However, it becomes essential that the holder containing the sample is as X-ray transparent as possible, because its “fingerprint” will be part of the X-ray pattern collected on each sample. Disposable, low background thin polymer films are commonly used to hold the sample in transmission measurements. In addition, it is common practice to regularly collect blanks (X-rays of the holder material itself) to ensure its signature does not change between different lots. Nevertheless, the extra effort required to operate instruments in transmission mode is well worth the improvement in data quality, especially when working with X-ray amorphous materials.

4 APPLICATIONS OF X-RAY DIFFRACTION IN DRUG DEVELOPMENT AND MANUFACTURING

XRD has a broad range of applications in various stages of drug development and manufacturing. This section will address many of the common XRPD uses from a practical standpoint. In the broadest terms, these applications can be divided between API characterization and identification. While there is some overlap in both categories, the former is more commonly applied during drug development (before the drug is on the market) while the latter is directed more toward manufacturing, regulatory aspects and intellectual property.

4.1 API Characterization

Guidelines from regulatory authorities regarding the need for characterization of a drug substance under development have been clearly stated. Below is an example relating to the issue of polymorphism (20):

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"Polymorphic forms of a drug substance can have different chemical and physical properties, including melting point, chemical reactivity, apparent solubility, dissolution rate, optical and mechanical properties, vapor pressure, and density. These properties can have a direct effect on the ability to process and/or manufacture the drug substance and the drug product, as well as on drug product stability, dissolution, and bioavailability. Thus, polymorphism can affect the quality, safety, and efficacy of the drug product."

While there are a number of methods to characterize polymorphs of a drug substance (21), the two broadly accepted methods of providing unequivocal proof of polymorphism recognized by the above source are single crystal X-ray diffraction and X-ray powder diffraction (20). Other techniques (e.g., thermal or spectroscopic methods) can be helpful in further characterizing drug products but only X-ray provides the necessary structural information to uniquely identify different polymorphs. Therefore, in early drug development, X-ray powder diffraction is often used as a primary experimental technique and a means of differentiating between the experimentally generated materials. Fully characterizing any material requires the use of complementary techniques (thermal or spectroscopic) but X-ray is typically done first because it is fast, non-destructive, requires little material, and provides the necessary structural information.

Synchrotron X-ray diffraction has frequently been used to characterize pharmaceutical materials in applications where additional sensitivity not provided by laboratory X-ray diffractometers may be required (e.g., crystallization monitoring (22,23)). The tradeoff is the greater expense and time investment typically associated with such measurements. Since such applications tend to be specialized, this section will focus primarily on laboratory XRPD methods.

4.1.1 Qualitative Analysis of Materials (Phase Identification) Every structurally different crystalline material will exhibit a unique XRPD pattern upon analysis (14). Therefore, the use of XRPD for phase identification was recognized early and remains the most common application of XRPD to pharmaceuticals. This so-called qualitative analysis typically refers either to the initial characterization of material previously not analyzed by XRPD or to the identification of a phase or phases in a sample of material by comparison to reference patterns. Reference patterns are previously collected XRPD patterns of the same material. Where available, XRPD patterns calculated from, for example, single crystal structures can be substituted but one should remember that the temperature at which the pattern is calculated can have a significant effect on the calculated XRPD profile. When dealing with mixtures of phases, qualitative analysis can provide an estimate of the relative proportions of different phases in the sample, usually based on the comparison of peak intensities for characteristic peaks of the different phases. Due to sample artifacts such as preferred orientation and poor particle statistics, this type of analysis should never be confused with quantitative analysis of mixtures, addressed later in this chapter. Databases of known XRPD patterns for various pharmaceutical materials are published annually by the Centre for Diffraction Data (ICDD) and the Cambridge Crystallographic Data Centre (CSD).

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XRPD patterns are typically compared by overlaying and aligning the data from different samples. This procedure is typically done electronically, either using the software provided by the XRPD instrument vendor or using custom-developed software. The primary assessment might include determining whether each sample is X-ray amorphous or crystalline based on the absence or presence of crystalline peaks, respectively. When comparing XRPD data of crystalline samples, one notes any differences in peak positions (to within a certain precision, e.g., $0.1^\circ 2\theta$ (24)) which correspond to structural differences between the samples. Intensities are generally not relied upon for qualitative analysis due to previously mentioned instrument and sample artifacts, although they have to be used to some degree to allocate the peak positions (based on local maxima).

It is not uncommon for two patterns to share some but not all of the peak positions. This can be a coincidence or it can be due to one of the samples being a mixture of multiple phases, including the phase in the other sample. Experience and data from complementary experimental techniques are needed to resolve such ambiguous cases. It should also be noted that, at higher 2θ values, peaks of most organic materials become considerably overlapped and determining their exact positions becomes difficult. Therefore, free-standing peaks at low angles are the primary means of differentiating structures and XRPD data above approximately $30^\circ 2\theta$ are rarely useful for qualitative analysis.

Figure 8 shows XRPD patterns of two crystalline polymorphs of sulfamerazine. The patterns in Fig. 8 were collected from material crystallized in glass capillaries during a polymorphism screen. A polymorphism screen is typically run early in the

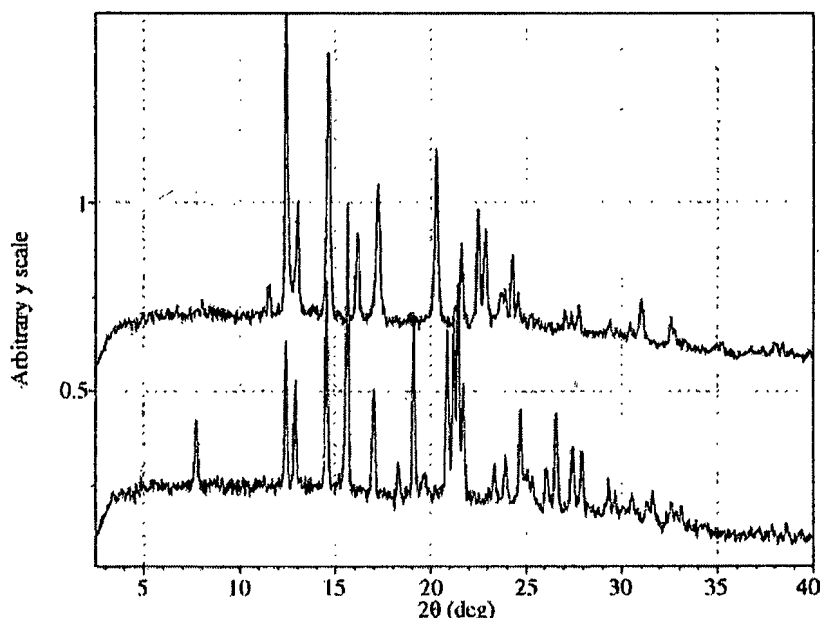


FIGURE 8 Two polymorphs of sulfamerazine. Patterns offset for clarity.

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drug development process to identify and (partially) characterize the different polymorphs of a drug substance. Assuming XRPD was the first analytical technique used on the samples, the data in Fig. 8 could be used to make a qualitative assessment regarding the probable nature of the material generated during crystallization experiments. Therefore, one could designate the first of the patterns crystalline "Pattern A" and the other crystalline "Pattern B," noting the sharp peaks and lack of diffuse halos as a sign of crystallinity and the structural differences, as evidenced by the different peak positions in each pattern. There is insufficient information at this stage to designate either pattern as a polymorph of the material (e.g., they could be a solvate, hydrate, or a mixture of two or more polymorphs). However, it is clear that both materials are crystalline and structurally different. Further characterization using for example, thermal methods (TGA, DSC) would confirm these materials are not solvates or mixtures but actual polymorphs and aid in determining the thermodynamically stable polymorph. XRPD provides information about the structure of materials, not thermodynamics, although variable-temperature XRPD has been used to study changes in structure at different temperatures.

One can envision a large number of different crystallization experiments (using different solvents or conditions) performed on the API, some possibly in automated fashion, with the resulting material characterized initially by XRPD. This is in fact a common approach to polymorphism, salt, and cocrystal screening and perhaps the most common application of XRPD in the drug development process. The latter two screens are usually performed when the polymorph(s) of the drug candidate itself are not sufficiently bioavailable, in an effort to produce a formulation that addresses the bioavailability problem. An XRPD pattern is taken of the API and the guest material (e.g. acid) and the mixture of the two. If a salt or cocrystal was formed, the XRPD pattern of the mixture should be more than just a sum of the reference patterns of the API and the guest.

Therefore, the first application for XRPD during drug development is typically to identify the materials generated using different experimental methodologies, often in automated high throughput screening environments (25–27). To simplify this pattern recognition problem that often involves hundreds or thousands of experimental data sets per screen, people have developed various computational approaches to recognize, sort, and classify unknown XRPD patterns, either through comparison to a known database of materials (28) or simply within the experimental set of unknown patterns (18, 29, 30). The latter often uses an approach called hierarchical clustering (31, 32).

XRPD data are often catalogued in databases using the so called Hanawalt system (33–34). In this system, the data are stored as d versus $1/\lambda_{\max}$ pairs. The use of d -space eliminates the need to specify the radiation source wavelength and allows comparison between laboratories using different instrumentation. A similar system is often used for intellectual property filings, as discussed later in this chapter.

However, as we shall see in following sections, there is considerable structural information available in a typical XRPD pattern that can be used to characterize the material. Making use of this information usually requires high quality laboratory data and the use of advanced computational methods.

4.1.2 Structural Analysis of Crystalline Forms XRPD patterns of crystalline materials have sharp peaks due to constructive interference of diffracted radiation. As mentioned previously, XRPD patterns can be used to identify particular substances by comparison with reference patterns, analogous to the use of fingerprints to identify people. For example, the experimental XRPD pattern of Mannitol in Fig. 9 (bottom pattern) compares best with the simulated pattern of the beta form (second pattern) indicating that the sample is likely form beta, and not form alpha or delta. The positions of the reflections in XRPD patterns are functions of the size and shape of the crystallographic unit cell. Reflection intensities are functions of the atomic positions within the unit cell. A variety of instrumental and sample preparation artifacts influence the intensities, and to a lesser extent, the positions of the reflections. A first step in the further characterization of an XRPD pattern of a crystalline sample is to rationalize the reflection positions using a process called indexing.

4.1.2.1 Indexing Indexing is the process of determining the size and shape of the unit cell given the peak positions in a diffraction pattern. The term gets its name from the assignment of Miller index labels to individual peaks. For most applications, the index labels are less important than are the unit cell length and angle parameters that provide the link between crystal properties and the diffraction pattern.

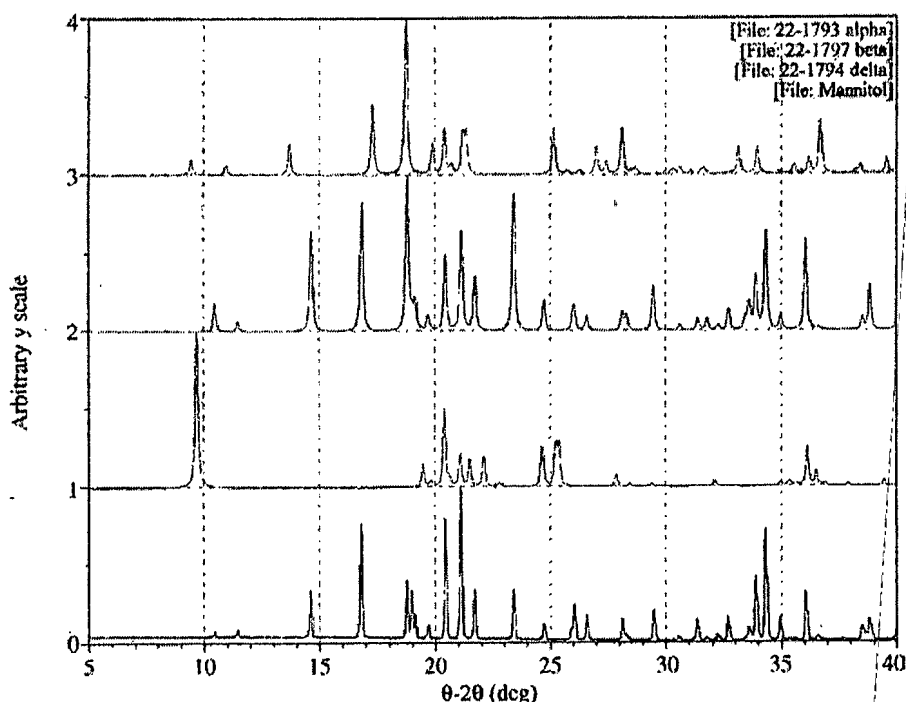


FIGURE 9 Comparison of simulated (top three) and measured (bottom) XRPD patterns of Mannitol. The legend provides ICDD reference codes and form designations for the simulated patterns.

Indexing plays a role in single crystal and powder diffraction pattern analysis, but there are qualitative differences between indexing single crystal and powder diffraction data. For single crystal diffraction, reflections are recorded as a function of the three-dimensional orientation of a crystal relative to the incident radiation. Each reflection in single crystal diffraction corresponds to a solution of the Laue equations, or equivalently the vector form of the Bragg equation. For powder diffraction, the (ideally) isotropic distribution of particle orientations leads to cylindrical symmetry in the diffracted radiation. Therefore, each reflection in powder diffraction corresponds to a solution of the scalar form of the Bragg equation (35). As a result of the loss of information regarding the relative orientations of diffracting crystallites, indexing of XRPD data is more challenging than single crystal diffraction data.

There are a variety of methods available for indexing XRPD patterns, some of which are in the public domain and others that are sold commercially. Examples include ITO (36), TREOR (37), N-TREOR (38), singular value decomposition (39), X-CELL (40), and DICVOL (41). The methods differ in their methods for identifying and refining potential solutions, ability to determine low symmetry solutions, efficiency, and sensitivity to extraneous and/or missing peaks. Development of novel methods and refinement of existing methods continues to be an area of active research. When applying these automated methods, it is important to be aware that multiple solutions can appear to adequately index a XRPD pattern. These degenerate solutions are called lattice metric singularities (42). In such cases, it is common practice to accept the higher symmetry solution unless there is other evidence supporting the lower symmetry solution.

Successful XRPD indexing serves several purposes. If all of the peaks in a pattern are indexed using a single unit cell, this is strong evidence that the sample contains a single crystalline phase. Given the indexing solution, the unit cell volume may be calculated directly. The difference in molar volume between an anhydrous crystal form and the molar volume of hydrates, solvates, and co-crystals can be useful for determining their stoichiometries. If the chemical composition of a crystal is known, then indexing provides the means of determining very accurate true densities. Indexing is also a robust description of a crystalline form. Common practice is to characterize forms using a list of XRPD peak positions for selected intense peaks, but the intensities of individual peaks are sensitive to changes in temperature, defect density, and preferred orientation effects as a result of sample preparation. Also, the positions of peaks are sensitive to temperature, strain, and changes in hydration state. Although unit cell parameters change as a result of these effects, the changes are less pronounced when comparing indexing solutions than when comparing peak lists based on particular selection criteria. Indexing can be used to show that a XRPD pattern is consistent with the structure of a crystal determined via single crystal diffraction. Also, indexing is a preliminary step for other analyses including structure determination from XRPD data and Rietveld refinement.

Data Not all XRPD patterns are amenable to indexing. XRPD patterns must have sufficient signal-to-noise and peak resolution to provide enough peak positions to uniquely define the indexing solution. Signal-to-noise can be improved by eliminating sources of diffuse scattering such as glass sample holders and by collecting the pattern

for a longer time. Peak width is a convolution of instrumental and sample contributions. Therefore, a diffractometer with adequate monochromatization is necessary, but not sufficient to ensure good peak resolution. Samples containing nanoscale crystalline regions or defective crystals display broadened peaks in their XRPD patterns. In the extreme, these broadened peaks can overlap and produce a pattern that appears to contain only amorphous halos as discussed elsewhere in this chapter.

Wilson showed that centrosymmetric crystal structures (those containing an inversion center) are more likely to have very weak and very strong reflections than are non-centrosymmetric crystal structures (lacking an inversion center) which tend to have a narrower distribution of peak intensities (43). Ordered crystals containing only one enantiomer (or diastereomer) of a chiral molecule cannot crystallize in space groups with mirror planes, glide planes, or inversion centers since those operations change the chirality of the molecule. Therefore, crystals containing non-chiral molecules or racemic mixtures of chiral molecules may adopt a centrosymmetric structure whose XRPD pattern has a large fraction of very weak reflections. Since these weak reflections are often important for successful indexing of the XRPD pattern, care should be taken to optimize peak sensitivity when collecting XRPD data for indexing, particularly for non-chiral molecules or racemic mixtures. This can be accomplished by reducing sources of background scattering and by increased collection times, for example.

The bottom XRPD pattern in Fig. 9 is an experimental XRPD pattern of Mannitol, form beta. Since Mannitol is chiral, it is expected to crystallize in a non-centrosymmetric structure and the corresponding XRPD pattern should have relatively few extremely weak or strong reflections. There is relatively little diffuse background scattering and the signal-to-noise ratio appears to be very good in the pattern. Also, the peaks are quite sharp leading to good peak resolution. All of these observations suggest that the XRPD pattern is amenable to indexing under the assumption that the pattern represents a single crystalline phase.

Results A successful indexing solution for the Mannitol XRPD pattern from Fig. 9 is illustrated in Fig. 10. The bars in Fig. 10 correspond to positions of allowed reflections based on Bragg's Law. All of the observed peaks in the XRPD pattern are indexed by one or more of the allowed reflections. Also, there are relatively few allowed reflections that are not observed. In some cases, the reflections are too weak to be evident at the scale shown in Fig. 10, but are evident using an expanded scale. If the allowed reflections did not account for all of the observed reflections, then the solution

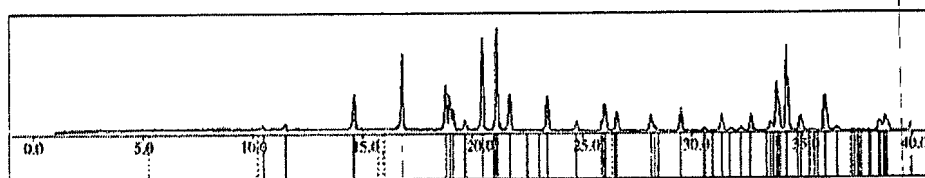


FIGURE 10 Indexed XRPD pattern of Mannitol form beta. Green bars indicate allowed reflections based on the unit cell dimensions and assigned space group $P2_12_12_1$ (no. 19). Dashed bars indicate extinct reflections.

would have been rejected as unable to fully describe the observed pattern. If there were a large number of allowed but unobserved peaks, then the solution would be rejected in favour of a smaller volume and/or higher symmetry solution. Since the illustrated solution falls into neither category, it is accepted as a good description of the observed pattern. This confirms that the XRPD pattern is of a single crystalline phase.

There are some allowed reflections, marked with dashed bars in Fig. 10, that are not observed. These systematic extinctions are the result of space group symmetry elements of the contents of the unit cell. Systematic extinctions are the result of centered unit cells, screw axes, and/or glide planes. Rules indicating which reflections are extinct based on their Miller indices are tabulated (35). Given the observed systematic extinctions in a pattern, an extinction symbol may be assigned (44). In the illustrated case, the extinction symbol ($P2_12_12_1$) corresponds to only one space group, $P2_12_12_1$ (no. 19). In many other cases, there are two or more space groups corresponding to the observed extinction symbol and additional analysis is needed to fully determine the space group.

The solution illustrated in Fig. 10 is orthorhombic with length parameters given in Table 2. Given the length parameters, the unit cell volume is readily calculated. The number of asymmetric units in the unit cell for a given space group is tabulated in Reference (44). For space group $P2_12_12_1$ there are four asymmetric units in the unit cell ($Z=4$). Assuming that there is one Mannitol molecule per asymmetric unit ($Z'=1$), then the molar volume and density are readily calculated. The density is a bit higher than typical organic molecules, but is as expected for sugars such as Mannitol. A reasonable density confirms that the correct number of molecules per asymmetric unit was assumed.

Having successfully indexed the XRPD pattern, determined the extinction symbol, and calculated the density, the analysis of peak positions in the Mannitol XRPD pattern is complete. Additional information regarding the Mannitol sample can be derived from the peak shapes and intensities using the Rietveld method.

TABLE 2 Indexing Results and Derived Quantities

Substance and Form	Mannitol Form Beta
Composition	$C_6H_{14}O_6$
MW (amu/molecule)	182.172
Family and space group	Orthorhombic $P2_12_12_1$ (no. 19)
a (Å)	8.6790
b (Å)	16.8980
c (Å)	5.5502
α (deg)	90
β (deg)	90
γ (deg)	90
V (Å ³)	813.65
Z' (molecules/unit)	1
Z (units/cell)	4
V/Z (Å ³ /unit)	203.41
ρ (g/cm ³)	1.487

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4.1.2.2 Rietveld Analysis The Rietveld method is a computational tool for extracting structural and microstructural information about a crystalline solid from its powder-diffraction pattern. This computational method was pioneered by Hugo Rietveld in the late 1960s (45, 46). The ability to obtain structural information from polycrystalline materials from which a crystal suitable for single-crystal analysis was either unavailable or practically impossible to obtain was a significant achievement. Initially the method was mostly used to refine the atomic positions and lattice parameters of crystal structures. Use of the Rietveld method proliferated with the increased accessibility of digital data in the 1980s and is now routinely used for quantitative analysis of phase mixtures for a wide range of materials and applications from cement and minerals to pharmaceuticals. This method is also used to characterize crystal defects and the microstructural properties of polycrystalline solids, such as preferred orientation, microstrain, and crystal size.

The Rietveld method computes a powder pattern using information describing the crystal structure and the instrumental technique used to collect the diffraction pattern. An iterative algorithm refines the structural and instrumental parameters used in the computation to minimize the difference (residual) between the observed and calculated diffraction intensity at each scattering angle (2θ). The success of a Rietveld analysis depends on building an appropriate model for the algorithm so the best fit (global minimum) to the experimental pattern can be efficiently discovered while avoiding false minima. Many freeware computer programs available for Rietveld analysis are listed and described on the website (47); many programs are also commercially available. To set up a model for a system of interest and obtain reliable information, each program requires a skilled user with understanding of diffraction theory and experiment. A recommended way to learn and assess the reliability of a Rietveld program is by refining patterns of well-characterized powders. α Al_2O_3 NIST SRM 676 is a very good standard because it has no preferred orientation, two refinable atomic positions determined by single-crystal analysis (48), and two lattice parameters. The accuracy of the measured intensity and 2θ combined with the skill of the Rietveld analyst and the accuracy of the Rietveld refinement algorithm determines the accuracy of the refined parameters. The applicable theoretical corrections used to compute the diffraction pattern for a particular data-collection technique require the user to judiciously construct the appropriate models (49, 50).

The minimum information needed to calculate a diffraction pattern is the atomic positions, space group, and lattice parameters of the crystal structure. The Rietveld program calculates the position and intensity of the diffraction peaks and assigns them a 2θ -dependent profile determined by the instrumental model. The parameters describing the profile are obtained from the results of a Rietveld analysis of a pattern of NIST SRM 660a LaB6 profile standard. This standard can also be used to check the accuracy of the lattice-parameter refinement and to refine the wavelength intensity ratio if more than one X-ray wavelength was used for data collection. For example, the ratio of Cu $\text{K}\alpha_2$ to $\text{K}\alpha_1$ is ~ 0.5 and can vary several hundredths depending on the type and alignment of the monochromator of the instrument used to collect the powder pattern. Standardizing the profile function allows microstrain and size (51) to be more

accurately refined as well as improving the fit of the calculated pattern to the experimental pattern.

Several quantitative and qualitative ways of assessing the "goodness of fit" between the experimental and calculated patterns are available. The qualitative assessment is a careful visual examination of the fit and the residual intensity. Visual assessment is essential and compliments the quantitative measures of the fit. The quantitative measures of the fit include the weighted pattern residual (R_w) and the ratio of R_w to the expected residual (R_w/R_{exp}). This ratio indicates how close the fit is to the theoretically best residual calculated using the number of data points in the observed pattern, the number of refined variables, and the uncertainty of the observed intensity intrinsic to counting photons.

The refinement is usually done in several stages (52) with only some of the refinable parameters being refined in each stage. The scale factor for the incident-beam intensity is always refined, and the background is usually refined in the first stage. The background is best minimized in the experimental pattern by use of antiscatter slits and helium; reducing background also improves the detection limit. Background is usually modeled with a polynomial and, if the background cannot be adequately fit, it can be artificially minimized by subtracting an appropriate blank pattern collected with identical collection parameters as the specimen of interest. Subsequent stages depend on the goal of interest. None of the refinable parameters will usually be refined well the first time; therefore, several iterations are used to find the global minimum.

A successful Rietveld refinement requires the user to know if the pattern of interest displays artifacts from inadequate orientation statistics (OS) and preferred orientation. These artifacts affect relative peak intensities and can generate significantly inaccurate structure refinements. If preferred orientation is well understood, it can be corrected by a variety of models available in many Rietveld programs. OS artifacts are manifest in random fluctuations of relative intensity for each preparation of the sample and cannot be corrected. The spiky peaks generated by OS artifacts can be difficult to detect by visual examination of the one-dimensional XRPD pattern. Fortunately, they can be readily detected by collecting a two-dimensional pattern with an area detector. If the Debye rings in the two-dimensional pattern are spotty, then OS is a potential problem depending on how the pattern of interest is collected. Both PO and OS artifacts can be practically eliminated by collecting the pattern in transmission geometry using a Gandolfi spinner and by comminution of the sample. Unfortunately comminution is often not a viable option because many samples, especially organics, are susceptible to stress-induced phase transformation.

We provide an example of the limitations of refining a pattern displaying a slight amount of PO. Rietveld refinement using Maud (version 1.999) was applied to a pattern of β mannitol collected with a laboratory diffractometer in transmission geometry. To correct PO we used a March-Dollase model on the 130 hkl (Fig. 11). The atomic positions were compared to those from a single-crystal structure determination by Kaminsky et al. (53). The thermal parameters were bound to be the same for all atoms and set to be isotropic; hydrogen was not included. Without using PO corrections, the fit to the measured pattern was poor (Fig. 12).

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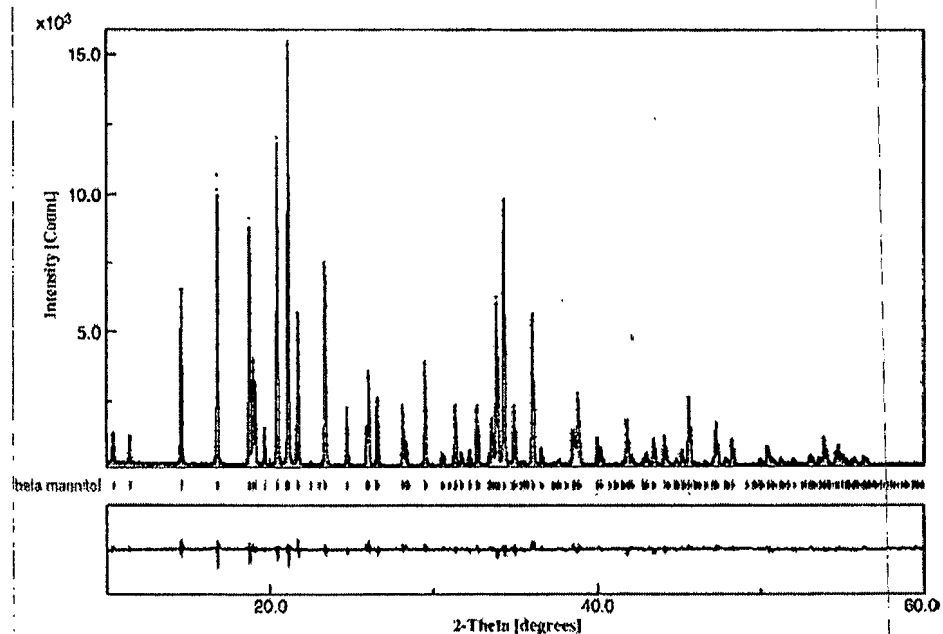


FIGURE 11 Overlay of a Rietveld refinement of β mannitol with $R_w = 7.19\%$ and $R_w/R_o = 1.6$ used to investigate the use of spherical harmonics to facilitate the refinement of the lattice parameters and thermal factor.

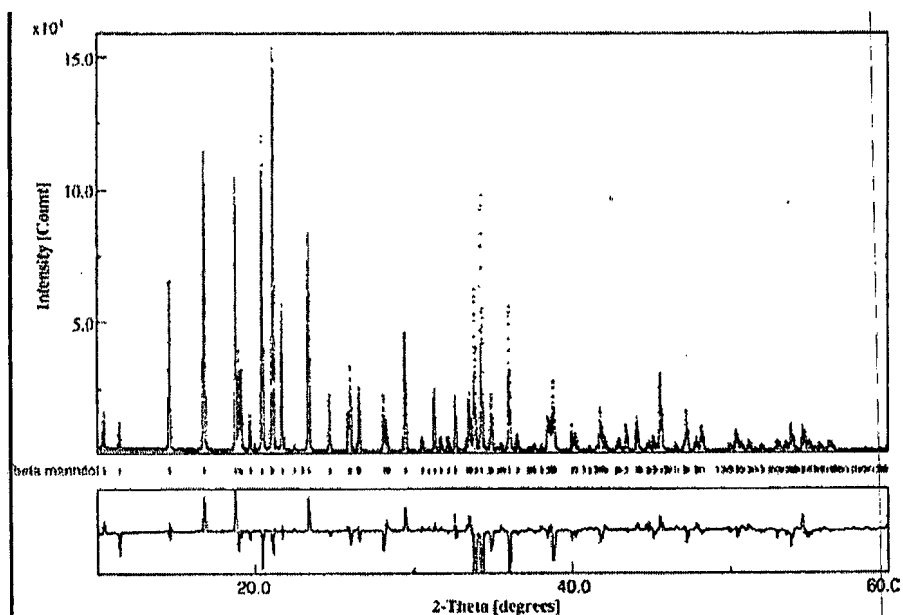


FIGURE 12 Rietveld refinement of β mannitol without spherical harmonics, $R_w = 26.3\%$ and $R_w/R_o = 5.9$.

TABLE 3 Bond Distances for β -Mannitol from a Rietveld Refinement of a Pattern with Orientation-Statistics Artifacts Compared to Those from a Single-Crystal Analysis by Kaminsky et al. (53) and a Rietveld Refinement by Botez et al. (54)

Bond Distance	Kaminsky et al. 1997 (Å)	Botez et al. 2003 (Å)	Difference (Å)	This Study (Å)	Difference (Å)
C1-C2	1.517 (2)	1.545 (17)	0.028	1.585	0.068
C2-C3	1.539(2)	1.546 (15)	0.007	1.543	0.004
C3-C4	1.523 (2)	1.515 (16)	-0.008	1.555	0.032
C4-C5	1.539 (2)	1.592 (14)	0.053*	1.618	0.079
C5-C6	1.521 (2)	1.552 (16)	0.031	1.569	0.048
C1-O1	1.425(2)	1.416 (17)	-0.016	1.485	0.053
C2-O2	1.440 (2)	1.443 (16)	0.016	1.444	0.017
C3-O3	1.433(2)	1.390 (17)	-0.042	1.431	-0.001
C4-O4	1.448(2)	1.434 (15)	-0.004	1.356	-0.082*
C5-O5	1.445(2)	1.439 (16)	0.011	1.461	0.033
C6-O6	1.436(2)	1.431 (18)	0.004	1.460	0.033
Standard deviation			0.026	n/a	0.040
Sum.			0.080	n/a	0.284

*Maximum deviation.

The bond distances, Table 3, from our refinement had a 0.04 Å standard deviation relative to the single-crystal values and a maximum absolute error as high as 0.08 Å. This uncertainty is ten times larger than the uncertainty of the single-crystal bond distances and slightly higher than those refined by Botez et al. (54) using a pattern collected with a synchrotron. Our bond distances also have a positive bias. These relatively high uncertainties reflect the intensity inaccuracies from not completely correcting the PO. The inaccuracies of atomic positions will increase as the magnitude of PO and OS artifacts increase. The calculated bond angles also will be more significantly more uncertain than the reliable single-crystal values.

The magnitude of thermal displacements above those commonly measured by single-crystal analysis indicates the amount of static-displacement defects in the crystal. The refined thermal parameter (B) was 2.4 Å^2 , and the single-crystal values for carbon and oxygen ranged from 1.64 to 2.81 Å^2 . This result indicates the 2θ -dependent intensity corrections provided by the Debye-Scherrer geometry and the curved position-sensitive detector models are reasonably accurate. The intensity fluctuations from slight PO are not expected to significantly affect the thermal parameter if enough of the pattern was refined, but, depending on the magnitude of the PO artifacts, a reasonably accurate B value might not always be obtained unless an accurate PO correction is applied.

The orthorhombic lattice parameters were refined to $a = 8.6790 \text{ Å}$, $b = 16.8980 \text{ Å}$, and $c = 5.5502 \text{ Å}$, and the zero offset was 0.0004° . These lattice parameters are within 0.01% of those refined by Botez et al. (54). The negligible offset indicates the diffractometer was very well aligned.

The beginning Rietveld analyst must have a good understanding of experimental and theoretical diffraction physics and the Rietveld model to assess the goodness of fit. The β mannitol example described here demonstrates a fairly good fit to the observed

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pattern using PO corrections does not necessarily mean the refined parameters are reliable and accurate; the bond distance and angles must be compared to known ranges from reliable single-crystal determinations to see if the results are reasonable. Values of bond distance and angle for moieties on organic molecules can be found in the Cambridge Crystal Structure database. Although patterns containing intensity artifacts can be analyzed using Rietveld analysis to provide useful structural information, the use of appropriate data-collection techniques is always the best way to obtain the most reliable results.

4.1.3 Structural Analysis of Disordered Crystalline Forms Highly crystalline forms of APIs are preferred in the drug development process because of their high level of purity and resistance to physical and chemical instabilities under ambient conditions (34). Unfortunately, most organic crystals are generally imperfect, containing different types of defects in the structure of the crystal lattice (4). The defective regions in a crystal introduce disorder into what is otherwise an ordered system, and correspond to sites with higher levels of energy in the solid (55). In many cases, defects can eventually lead to the formation of glass or amorphous (supercooled liquid) materials. Defects are typically formed through kinetic processes, for example, milling or dehydration, as opposed to thermodynamic processes. Whereas defects can often be reversed, the formation of amorphous material creates a different solid state that can agglomerate or recrystallize into a different crystalline form. Pharmaceutical interest in the study of defects arises from the demonstrated role that these high energy sites can play in affecting a number of important physical and chemical phenomena. The presence of defects can, for example, produce higher dissolution rates (56, 57), greater chemical instability (58, 59), altered mechanical properties (60–62), and enhanced hygroscopicity (63, 64).

XRPD combined with computational methods can be used to study crystalline defects (4, 15), even quantitate kinetic disorder parameters (e.g., crystal strain). Random defects within a crystal structure will generate diffuse X-ray scattering similar to X-ray amorphous scattering, though with halo positions and widths that are typically different from those measured from the amorphous (supercooled liquid) phase (15). This rule can be broken when defects in the material form a kinetic glass but it is possible to monitor the agglomeration of defects starting with perfect crystals all the way through glass, using XRPD. Such a study was reported in Reference (15) and is summarized below as an example application of XRPD to the study of crystalline defects.

In this study, defects were introduced into Raffinose pentahydrate through dehydration over time. XRPD measurements were taken at regular time intervals, Fig. 13, to monitor per cent crystallinity (65) and total diffraction methodology was used to model both the coherent long range crystalline contributions and incoherent short range disorder components together as a single system (66, 67). Rietveld methods were used for structure modeling (68, 69) and to track the structural phase changes in crystalline material. In addition, methods built around the use of the Pair Distribution Function (PDF) (8, 11, 70–75), $G(r)$ in Eq. 3, were employed to confirm the Rietveld results. Finally, it was shown that the introduction of defects through

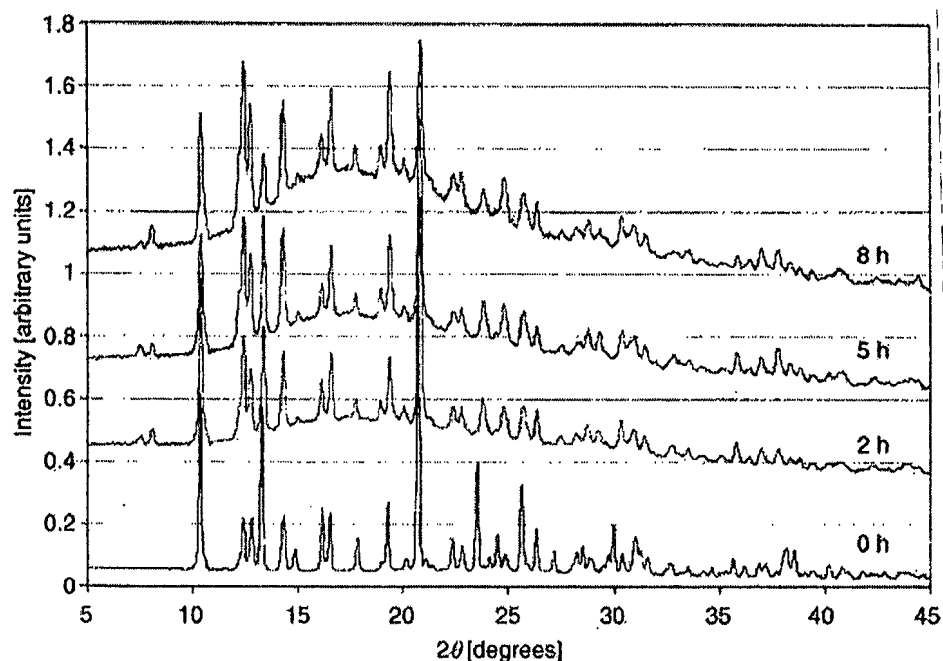


FIGURE 13 XRPD patterns collected in transmission mode for raffinose pentahydrate dried at 60°C under vacuum for (from bottom to top): 0, 2, 5, and 8 h, respectively.

dehydration of an organic crystal hydrate eventually leads to the formation of amorphous material.

$$G(r) = 4\pi r[\rho(r) - \rho_0] \quad (3)$$

Where ρ_0 is the average number density of the structure and $\rho(r)$ is the atom pair density for X-ray scattering (76) (Eq. 4).

$$\rho(r) = \left(\frac{1}{4}\pi r^2\right) \sum_{p,q} \left(\frac{f_p f_q}{\langle f \rangle^2}\right) \delta(r - r_{pq}) \quad (4)$$

The terms in Eq. 4 include $f_{p,q}$, the individual atomic form factors; $\langle f \rangle$, the mean atomic form factor of the structure; and the distance r_{pq} , corresponding to the atom pair separation. $\rho(r)$ represents the probability of finding an atom pair separated by the distance r , weighted by the atomic form factors and averaged over all atom pairs in the structure. The calculation needs to be evaluated over a crystal structure large enough to give the atom pair relationships for a distance of interest.

The PDF is a generally useful computational tool when analyzing X-ray amorphous or disordered systems. PDF analysis unlocks information present (but not obvious) in XRPD patterns, providing a measure of inter-atomic distances that define a solid form (70). From these distances, one can draw conclusions regarding the relationship between ordered and disordered forms of a material. By convention (71),

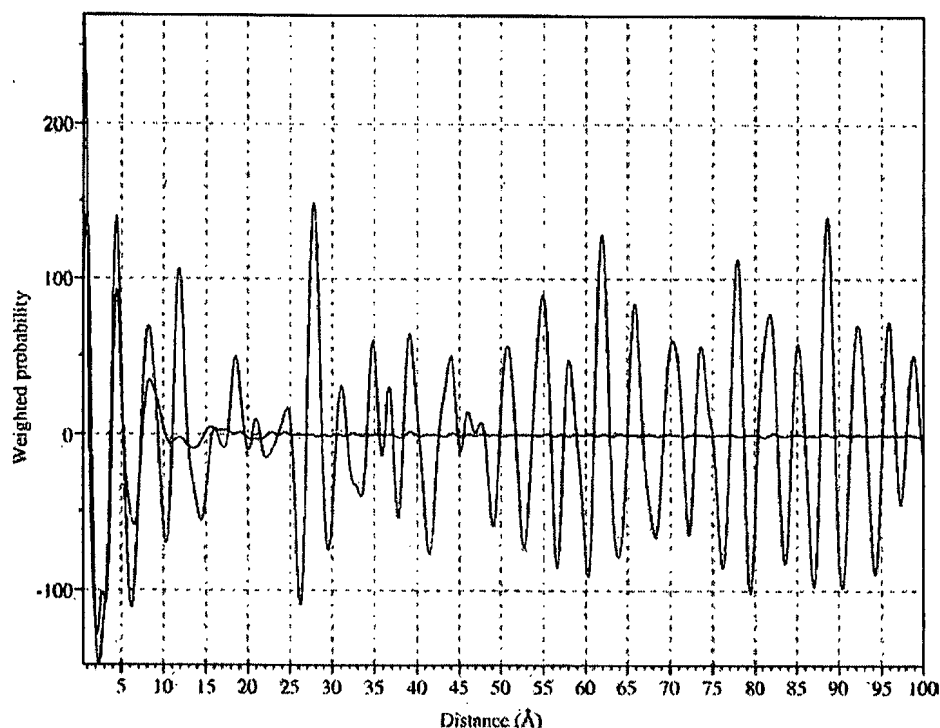


FIGURE 14 PDF traces of crystalline (lighter, with many peaks) and amorphous (darker, with few initial peaks) felodipine. The crystalline sample shows long-range order as evidenced by peaks in the PDF up to and exceeding 100 Å. The amorphous sample shows only short-range order with PDF peaks disappearing after approximately 20 Å. Note that both samples have similar short-range order with peaks in the PDF overlapping below 10 Å.

the PDF trace is typically plotted as distance (in Å) vs. (weighted) probability of finding two atoms with that separation. Peaks in the PDF correspond to inter-atomic distances that are common to the material in question, with the product of the peak area and distance giving the number of atom units with that specific separation. Figure 14 illustrates a PDF trace of amorphous felodipine superimposed against a PDF trace of crystalline felodipine. Both traces show similar short-range order (below 10 Å) but whereas the amorphous trace has no peaks in the PDF trace above approximately 20 Å (and therefore no long-range order), the crystalline trace contains peaks up to and exceeding 100 Å (indicating long-range order). Differences between defective crystalline (X-ray amorphous) and amorphous (supercooled liquid) materials can be seen in the PDFs calculated from XRPD data (15).

4.1.4 Structural Analysis of Organic Amorphous Materials Amorphous organic solids, also referred to as disordered systems (77, 78), are characterized by a lack of long-range order that is observed in crystalline structures (2). They are often described as supercooled liquids, turned solid by the removal of thermal energy or a solvent without inducing crystallization (65, 78). Many of the early stage drug candidates currently under development exhibit very poor aqueous solubility (2, 79).

Higher molecular weights than were common in the past and greater hydrophobicity are thought to contribute to the decrease in aqueous solubility of organic compounds. With data to suggest that the dissolution rate is the rate-limiting step in oral absorption, it is expected that reduced solubility will lead to reduced oral bioavailability (80).

Amorphous organic compounds tend to have significantly higher initial aqueous solubilities (non-equilibrium) and dissolution rates as well as greater compressibility and different hygroscopicity, as compared to crystalline forms of the same compound (65, 81). Therefore, in compounds where the crystalline forms are poorly soluble, the amorphous form often presents an attractive formulation option (82). Dozens of APIs and excipients currently on the market are listed as being amorphous (65). Because crystalline forms are more thermodynamically stable than amorphous forms, there is a driving force for crystallizing the amorphous state. Therefore, amorphous materials are inherently unstable (both physically and chemically) and, once crystallized, the material loses the solubility advantage provided by the amorphous form. The inherent instability coupled with a lack of developed characterization tools has limited the commercial potential of working with amorphous materials at present (65, 83).

A common method used by formulation scientists to overcome the instabilities associated with amorphous materials is to prepare a composition of the amorphous API with pharmaceutically acceptable excipients that provide a barrier to crystallization of an amorphous drug product upon storage. Such compositions are often referred to as "dispersions" or "solid dispersions." Stabilizers are often selected from a variety of polymers, a commonly used one being polyvinylpyrrolidone (PVP). Intimate mixing (mixing at the molecular level) between such excipients and the amorphous API is an important factor in compositions resistant to crystallization (84). To provide a barrier to structural changes, the excipient needs to be intimately mixed (e.g., in a solution) with the API, which is often difficult to accomplish or even identify.

As noted earlier, XRPD is a useful technique in the analysis of amorphous materials. In its simplest application, XRPD is used to identify X-ray amorphous materials and classify them separately from crystalline materials (18). It can also be used to further differentiate between (supercooled liquid) amorphous material and kinetic glassy material (15, 85) or to monitor the conversion of crystalline material into amorphous material upon processing (e.g., grinding) (85) (Fig. 15). In the latter application, note the steady peak broadening and loss of intensity at higher angles in the XRPD patterns, Fig. 15, as the material becomes progressively disordered. However, some materials may immediately collapse into a binary amorphous-crystalline mixture and eventually pure amorphous material, without any broadening of the crystalline peaks that would be indicative of disorder in the crystalline phase (85).

Finally, in the analysis of multi-component amorphous mixtures, XRPD along with computational methods (16) have emerged as techniques that can be used to identify the so-called "miscible" dispersions, as compared to the phase separated physical mixtures where intimate mixing was not achieved. XRPD is often accom-

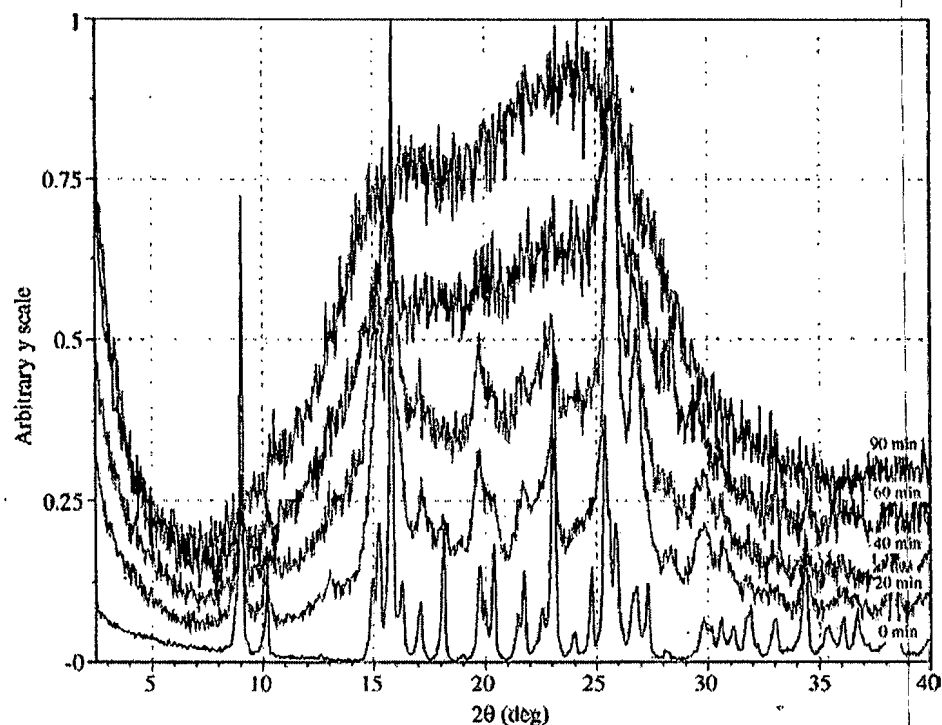


FIGURE 15 Monitoring of cryo-grinding experiments on piroxicam form II using XRPD. Bottom-to-top, starting pattern, after 20, 40, 60, 90 min of grinding, respectively.

panied by the use of complementary experimental techniques in this type of amorphous mixture analysis. Traditionally, thermal methods such as DSC have been used to measure glass transition temperatures when evaluating miscibility (86) and, while those methods have been shown to have limitations (16), their use is still recommended when analyzing amorphous mixtures (in addition to XRPD).

An example of this type of XRPD and computational analysis was reported in Reference (16). Solid dispersions of three different systems were analyzed initially using thermal measurements. Glass transition temperatures were identified from thermal data. For the system where two glass transition temperatures were identified, phase separation was indicated. The other two systems had a single glass transition temperature, suggesting miscibility and therefore stability to crystallization. XRPD data were collected on all three systems and the patterns were then analyzed using computational methods. Linear combinations of measured XRPD patterns and calculated PDFs were used to model the systems to determine the level of interaction between the two components in each system. The conclusions derived from thermal data were confirmed by XRPD for two of the three systems. However, the third system was identified as a so-called nano-suspension with domain sizes smaller than the resolution of thermal methods. Therefore, a prediction was made that this system is in fact phase separated despite the single glass transition temperature, and would recrystallize. This prediction was confirmed elsewhere in the literature (87).

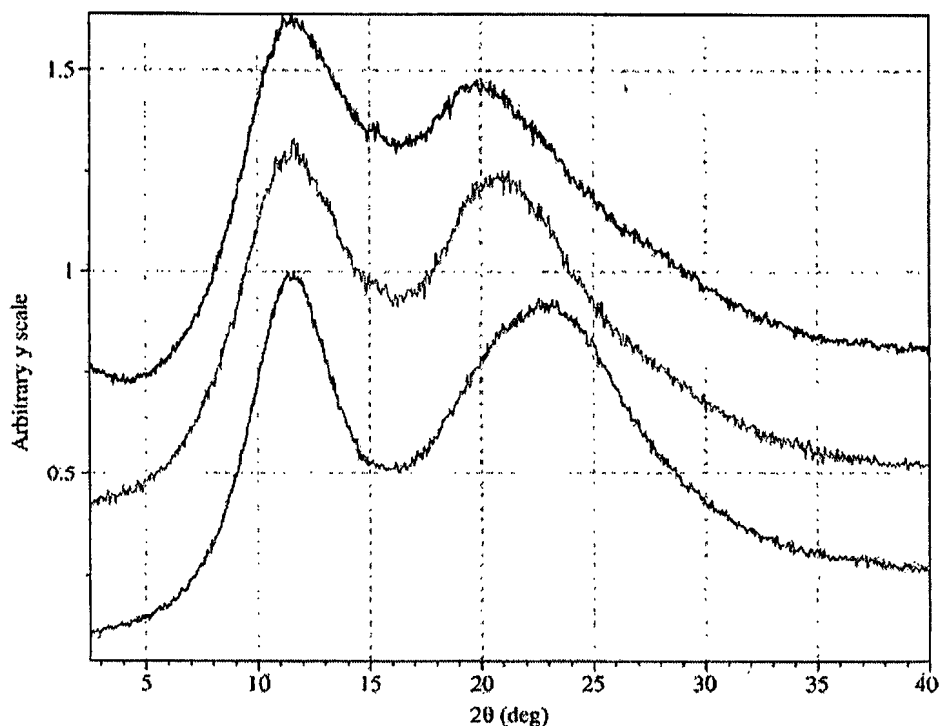


FIGURE 16 XRPD patterns of, top-to-bottom, amorphous PVP, a solid dispersion of PVP and felodipine (70% PVP, 30% felodipine by weight), and amorphous felodipine.

Figure 16 shows example XRPD patterns of a pharmaceutically acceptable excipient (PVP, top pattern), amorphous drug product (felodipine, bottom pattern), and a solid dispersion of the two (70% PVP and 30% felodipine by weight, middle pattern). Despite the lack of long-range order (and therefore crystalline peaks), one can observe clear differences in the structure of these amorphous materials from these XRPD patterns, using the position and width of the two broad halos in each pattern. For example, the second halo in the dispersion is located in between the second halos for the excipient and the drug product, suggesting it is a mixture of the two materials. Additional computational analysis could be applied (16) to determine the level of miscibility of the two components in the dispersion.

4.1.5 Characterization of Multi-Component Mixtures A common application for X-ray powder diffraction is in the characterization of multi-component mixtures. The technique is highly effective when used for this application provided the materials are structurally different (88) and fall within the limit of detection of XRPD. With good laboratory instrumentation and sample preparation methods and typical counting times, the limit of detection for XRPD can be on the order of 2–3% by weight, or even lower when detecting the presence of crystalline materials in an amorphous-crystalline mixture. In multi-component crystalline mixtures the limit of detection can become significantly worse, especially if the component being detected has no unique peaks as compared to

the other components in the mixture. The detection of low levels of amorphous material mixed with a predominately crystalline sample can be difficult and is a subject of ongoing research (65). XRPD can also be used to quantify the amount of (crystalline or amorphous) component in the mixture as the diffracted intensity in the pattern is related to the weight percentage of the component in the mixture.

4.1.5.1 Characterization of Crystalline Mixtures (Quantitative Analysis) Quantitative analysis of crystalline mixtures typically refers to the determination of the relative amounts of different phases in a sample containing multiple phases (i.e., in a mixture), using experimental XRPD data. In broad terms, quantitative methods can be separated into those requiring the use of internal or external standards and ones based around the use of the full diffraction pattern (5, 11, 14, 89, 90). The application of standards to this problem is not new, dating back to the 1930s (91). When using standards, it is essential to work with a randomly oriented fine powder specimen to avoid problems with preferred orientation or poor particle statistics and to be confident that the sample is representative of the material being analyzed (5, 14). In addition, there are a number of structure, instrument, and measurement-sensitive factors (14) that affect quantitative analysis.

The internal standard method (92, 93) (also called the RIR method, for reference intensity ratio) is the most general and the most commonly used of any XRPD quantitative phase analysis methods in the past. It requires that a known weight percentage of a standard material be homogeneously mixed with the sample whose phase composition one is trying to determine. The method is based on dividing the intensity of a line (peak) of the phase whose ratio in the composition is being determined by the intensity of the line for the internal standard used. As such, it is important to choose an internal standard that has peaks that do not overlap with any found in the mixture itself. Examples of standards include corundum, silicon, and SRM 676 (Al_2O_3) (5). The latter is particularly good at minimizing preferred orientation problems. For a detailed discussion on the RIR method see for example, (5, 14), in recent years it has been increasingly replaced by full pattern methods because of difficulties in ensuring random orientation of all components in the mixture.

The full pattern methods can be traced to quantitative work performed on cements in the 1960s (94–98). The same principles apply to pharmaceuticals with some modifications, the most important of which is the need to normalize the total diffracted intensity across different patterns before using the full pattern method. The simplest of such full pattern methods rely on fitting entire observed or calculated patterns of reference materials to an observed pattern of the mixture (5, 99). If not previously available, XRPD patterns of the reference (standard) materials must be first collected, meaning one must know the composition of the mixture beforehand. The references must be analyzed under the same conditions and instrumental settings as the mixture. When possible, the instrumental background and noise should be removed by data processing (100). Provided the same sample preparation techniques were used to

make the reference materials and the mixture(s), sample artifacts should not present as big a problem as for internal standard methods as the full pattern methods do not rely on single peaks. The weighted sum of the selected reference patterns is fit using least-squares minimization (e.g., (101)) to the unknown mixture. In this procedure, each point in the pattern is treated as an independent observation and a single weight is applied to each pattern, giving its ratio in the mixture. The best calculated fit is reported along with the weights used to obtain this pattern (5, 98, 99).

A second commonly used full pattern quantitative method is based on the use of the Rietveld method (5, 14, 102). In this method, the refinement is done by minimizing the sum of weighted, squared differences between measured and calculated intensities at every 2θ position in the pattern. The Rietveld method does require the knowledge of the crystal structure for every component (of interest) in the mixture. This method is applicable even in the presence of overlapping peak lines and is less sensitive to preferred orientation problems. However, the requirement to have at least approximate crystal structures of every component can be problematic for APIs under development. Some examples of Rietveld applications to quantitative analysis include (103–105). Freely available software, for example, TOPAS (106) or GSAS (107), can be used for Rietveld quantitative analysis.

Finally, novel quantitative approaches using full pattern fitting are still an active area of research. Recent examples include References (89, 108).

4.1.5.2 Characterization of Crystalline-Amorphous Mixtures (Per cent Crystallinity) Degree (or per cent) of crystallinity is perhaps the most common quantitative application of XRPD in the drug development process. When the amorphous phase is present or suspected, XRPD can be used to characterize the material and determine the ratio of crystalline to amorphous material in the sample. Most X-ray methods used for this purpose are based on total sample scattering (i.e., scattering from both the amorphous and crystalline phases). In addition, the total diffracted area in each measurement needs to be normalized between samples to account for example, weight or absorption differences across samples. Once the patterns are normalized, per cent crystallinity can be easily calculated as a fraction of crystalline content in the total diffracted intensity measured from a sample (109). Several methods exist to estimate the crystalline content in an XRPD pattern (65, 110):

- i. Measuring the integrated peak area under the crystalline peaks and dividing by the X-ray intensity scattered by the entire sample (65). This is a common approach that works well except when many overlapping crystalline peaks are present. Examples of this type of analysis can be found in, References (110–122).
- ii. Measuring the maximum intensity of a subset of characteristic peaks (109). This method can only be applied if all the samples are analyzed under the same conditions, there is no peak broadening due to disorder or particle size issues, and a reproducible ratio of intensity can be demonstrated for the chosen peaks across multiple samples. Typically, the use of internal standards is recommended for this method (65, 109) to ensure the above conditions are met. Such

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internal standards can include lithium fluoride, silicon metal powder, or zinc oxide. A variation on this method uses peak area rather than height (123). Examples of these types of studies include References (123–129).

- iii. Quantification of the amorphous content using a region where no crystalline peaks appear in the mixture pattern (130). Since the diffraction in such regions is caused by short-range ordering of amorphous materials, it can be used to estimate the amorphous content present in the sample. The method may be more robust and sensitive than peak intensity measurements (110) though it is limited to mixtures where crystalline peaks are not present in the entire range of amorphous diffraction.

Other methods for the determination of per cent crystallinity have been reported in literature, for example, using specialized instrument optics (131). If both amorphous and crystalline reference patterns are available, full pattern quantitative or semi-quantitative methods used in crystalline mixture analysis can be applied here as well. Such an example is shown in Fig. 17. Physical mixtures of amorphous and crystalline sucrose were prepared with known weight ratios (0%, 20%, 40%, 60%, 80%, and 100% crystalline). The pure amorphous and pure crystalline patterns were then linearly fitted using Brent minimization (101) to each mixture. A single weight was used per reference pattern (applied to all intensity points) and the sum of squared differences at each intensity point was used as the error metric. All patterns were first

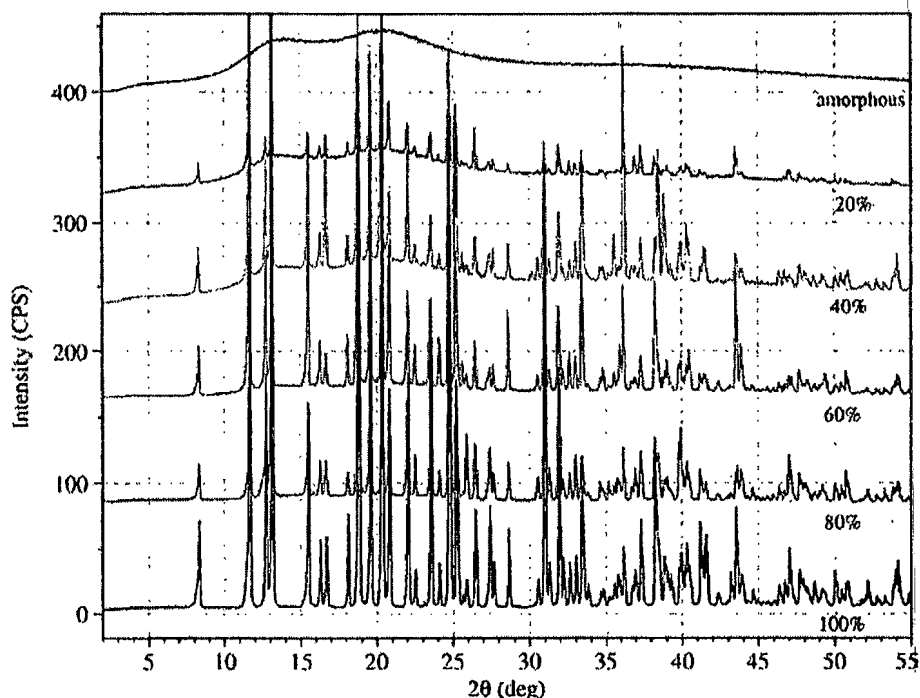


FIGURE 17 XRPD quantitative analysis of physical mixtures of amorphous and crystalline sucrose. Top-to-bottom, amorphous sucrose, 20, 40, 60, 80 and 100% crystalline sucrose.

normalized to same total diffracted intensity. The calculated per cent crystallinity values for the 20/40/60/80 mixtures were 17.2%, 40.7%, 64.4%, 84.3%, respectively. In practice, this approach coupled with good XRPD data consistently yields results within 5% of actual values for mixtures ranging from 10 to 90% crystalline.

4.1.5.3 Re-Crystallization of Amorphous Materials XRPD can also be used to monitor re-crystallization of amorphous materials. A typical application of this kind would be to characterize the extent of crystallinity during processing steps (e.g., scale-up of bulk material, formulation, manufacturing) or simply upon storage over the intended shelf life of the drug product, to ensure safety and efficacy (132). As noted earlier, amorphous materials will have different performance than crystalline counterparts (65,133), including for example sensitivity to moisture (134–136), reduced chemical stability (113,126,137), postcompression hardness (112), and enhanced dissolution rate (81,138).

Figure 18 shows the monitoring of recrystallization of amorphous felodipine by XRPD over a 7 day period. The top pattern is pure amorphous felodipine, the remaining patterns were collected on the same sample stored under ambient conditions (25°C and 50% RH) after a period of 2, 3, 5, and 7 days, respectively. The aforementioned computational methods were used to determine per cent crystallinity in each pattern, with the first and last patterns used as reference amorphous and crystalline material, respectively. Given those assumptions, the pattern collected after

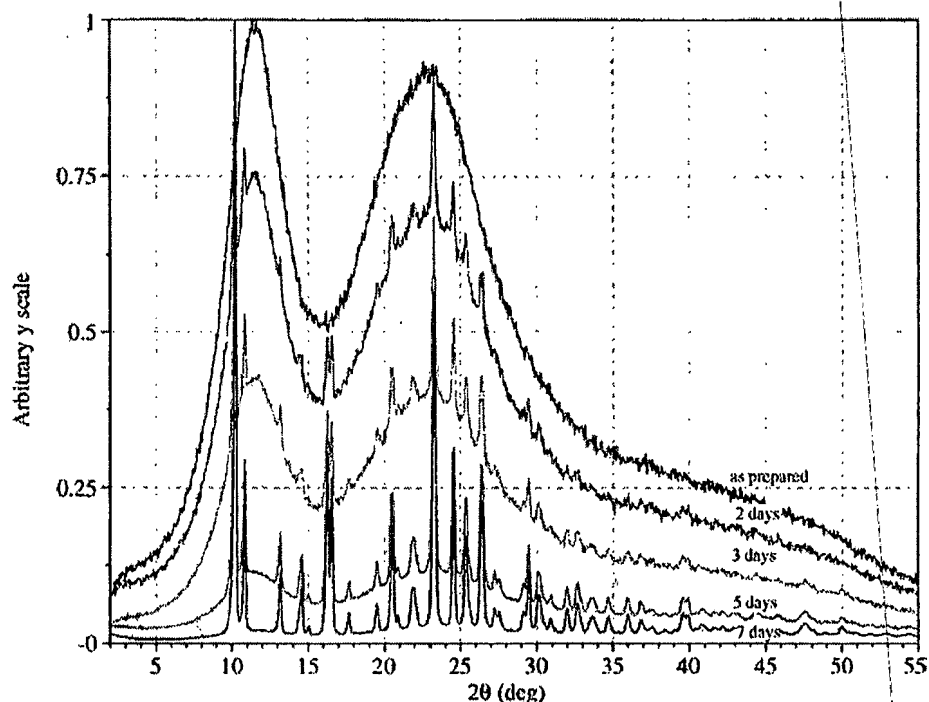


FIGURE 18 Recrystallization of amorphous felodipine monitored by XRPD. Top-to-bottom, amorphous felodipine, same sample after 2, 3, 5, and 7 days in ambient conditions, respectively.

2 days is approximately 5% crystalline, followed by 11% after 3 days and 45% after 5 days. After 7 days the sample is almost completely crystalline.

4.1.6 Regulatory Considerations The Food and Drug Administration's (FDA) New Drug Application (NDA) guideline (139) states that "appropriate" analytical procedures should be used to detect polymorphic, solvated (including hydrated), or amorphous forms of a drug substance. Likewise, the guideline states that it is the applicant's responsibility to control the crystal form of the drug substance. If bioavailability is affected by the change in crystal form, the applicant must demonstrate the suitability of the control methods. This highlights the importance of controlling the crystal form of the drug substance and, as shown in previous sections, the use of XRPD can provide essential structural information on crystalline and amorphous forms of a drug substance.

The ICH Q6A document (139) provides clear guidelines on how X-ray information should be used in the NDA. As noted in the section on API characterization, XRPD is one of two broadly accepted methods of providing unequivocal proof of polymorphism by the regulatory authorities (20), the other being single crystal X-ray diffraction. Characterization of the API by X-ray and/or some other method is required by the NDA guidelines as different solid forms may have different properties (20,140). An often-cited example is ritonavir (141), whose amorphous form is up to 20 times more bioavailable than the crystalline form, and which crystallized into a new less soluble form after 2 years on the market. XRPD is directly mentioned in the ICH decision trees as part of the drug application process, Fig. 19 (140,142). Some NDA submissions include XRPD methods on the drug substance.

It is often necessary to establish a specification or test (e.g., XRPD or IR) to ensure that the proper drug form is manufactured. Failure to do so may result in the manufacture of different polymorphs with different properties, potentially altering the dosage form performance (as in the case of ritonavir). Regulatory authorities are aware of such challenges and expect the manufacturer to demonstrate control over the

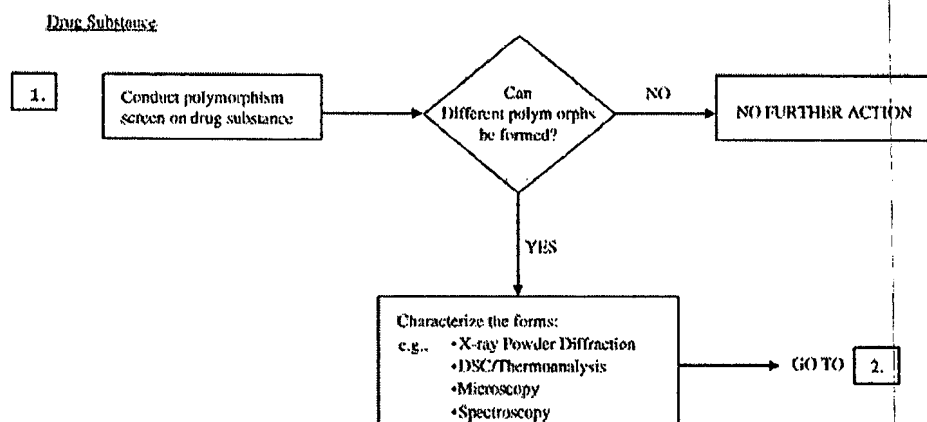


FIGURE 19 ICH Q6A decision tree directing characterization of the forms by X-ray powder diffraction (adapted by author from Reference (140)).

crystalline or amorphous form in the manufacturing process. Production scale-up requires a process be developed that can reproducibly make the desired polymorph. Where mixtures of forms cannot be avoided, quantitative control (e.g., using XRPD as outlined earlier) is typically required to ensure that the ratios between forms are maintained. Furthermore, this requirement may extend beyond the manufacturing process and through the retest date of the drug substance and potentially throughout the shelf life of the product, which can be a difficult requirement if the forms interconvert. Quantitative XRPD techniques are typically validated by following the ICH Q2A and Q2B guidance (139). It is therefore advantageous to select the most stable solid polymorph for production, determined using a process called polymorph screening in which XRPD plays a major role as outlined earlier. (The use of the most stable form would ensure that there would be no conversion into other forms).

Certain USP monographs require the use of XRPD on the drug substance. One example is the carbamazepine monograph which states that the X-ray diffraction pattern conforms to that of USP carbamazepine RS, similarly determined (140). The exact method of certification used by the USP in the reference standard is not known. In addition, USP recommends the precision to use when comparing peak positions between different XRPD patterns, that is, a peak in one XRPD pattern that falls within the USP-specified range of a peak in the other pattern is to be considered the same. That precision has varied from 0.1 to 0.2 °2 θ , depending on the year of USP issue (24).

XRPD has been identified as a unique tool for drug substance and/or product analysis that can directly contribute to FDA's "quality by design" initiative. In addition, this tool can help improve the time to market by providing information regarding the structure of the molecule early on in the development process. Finally, XRPD methods can be used to ensure the drug product and/or substance is consistent and has the same identity, purity, and potency (140).

4.2 API Identification

4.2.1 Analysis of a Drug Product XRD is frequently applied to the analysis of a drug product. A simple XRD method used for this purpose might involve the following steps (140):

1. Determination of the XRD patterns of all solid components in the drug product.
2. Selection of a region or regions in 2 θ where only the polymorph of interest (analyte) diffracts.
3. Preparation of reference physical mixtures containing all API forms as well as excipients.
4. Development of computational/mathematical methods.
5. Validation of the method following the ICH Q2A and Q2B methods.
6. Application of the method.

Chemometric (PLS) methods (143–146) may also be used to increase sensitivity and lower the detection limit. Such analytical XRD methods can have a variety of

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applications ranging from online process monitoring to support in litigation of drug product counter-fitting.

One example of the above approach that also includes quantitative analysis of the active ingredient is the XRD method developed by Suryanarayanan (147). The method was applied to intact tablets and required preparation of mixtures containing various weight fractions of the drug and excipient. The quantitative analysis relied on identifying integrated intensities of several diffraction lines for each compound and calculating their ratios as a function of the weight loading of the drug in the tablet. The reported error for the method was less than 10% for a drug loading of 40% weight-in-tablet.

4.2.2 Monitoring Effects of Processing and Manufacturing on the API XRD has been used to study the effects of processing and manufacturing on the solid form of an API. Many of the previously mentioned XRPD methods used for API characterization can also be applied for this purpose.

For example, the antiviral drug MCC-478, known to exist in several anhydrous polymorphic forms as well as a hydrate, was studied in tablet formulation using XRD (148). The XRD patterns of the tablets containing the various forms of the MCC-478 revealed at least one peak unique to each form—generally a prerequisite for this type of work. A semi-quantitative method was developed to characterize the physical form of the API in intact film-coated tablets despite the relatively low weight loading of the API (<20%) in the tablet that also contained a highly crystalline excipient, mannitol, in much greater proportion (60%). The use of this method confirmed that the aqueous film-coating process did not change the form of the API. Furthermore, the same method was used to verify that the API form remained stable (unchanged) over 6 months under accelerated aging conditions (40°C/75% RH). Therefore, the XRD method found application not only for process control during manufacturing, but also for the quality control of the final product.

With increased emphasis by regulatory authorities on understanding and controlling production processes, the implementation of online XRD methods as part of Process Analytical Technology (PAT) is gaining momentum (140). Production processes that are well understood and controlled are considered to be lower risk than those that have not been subject to PAT. It should be noted that spectroscopic methods (NIR, IR, Raman) are also frequently used to monitor polymorphic conversion during processing. Each method has its advantages and disadvantages but typically XRD methods are capable of analyzing the largest sample volume simultaneously and have been used extensively for this purpose in the cement, gypsum and catalyst industries.

An example application of XRD online monitoring was a study of polymorphic conversion during wet granulation (140,149). Wet granulation is a size enlargement process that uses a binder and water to form larger agglomerates, potentially causing phase transformations during the water addition step. Two enantiotropically related forms of flufenamic acid were monitored with a transition temperature of 42°C. XRD was able to identify the form present at various steps of the process.

Online XRD methods used to monitor crystallization of a drug substance have been the subject of extensive research (e.g., Reference (150)). Online processing cells have

been developed for the examination of temperature-dependant polymorphic changes in pharmaceutical materials using *in situ* XRD. Such cells can recirculate the crystallizing solution and monitor the changes by XRD.

4.2.3 Intellectual Property Considerations XRPD is frequently used to identify and/or characterize new materials in patent application filings. Typically, a list of XRPD peak positions is provided, listing 2θ and/or d -space position and relative (to maximum) intensity of crystalline peaks. Estimated experimental errors in position (2θ and/or d -space) may be provided, often adopted from the USP (24). It is important to remember that aforementioned instrumental and sample artifacts can and do affect peak positions and intensities. Therefore, providing strict limits on peak positions or relying on intensity information in patent claims yields data that are too narrowly defined. A skilled patent practitioner should therefore be consulted on how best to incorporate solid-state data into a patent application.

The XRPD peak listings may include a list of all observed peaks that can be visually identified above the noise level in the pattern (typically determined through an intensity threshold). However, since it is unlikely that all such peaks will be reproduced in every XRPD pattern of the material, a subset of low angle, non-overlapping peaks with strong intensity (10% or more of maximum observed peak intensity) may be included as peaks "representative" of the material. In this case, it is helpful to have access to XRPD data collected on more than one type of diffractometer geometry in order to assess the reproducibility of peak intensities, which can frequently be affected by for example, preferred orientation and poor particle statistics in a sample.

Where materials exhibit polymorphism and multiple forms are found, it is typical to provide a listing of characteristic (unique) peaks that differentiate one form of a material from all the other known forms of the same material. For different salts or co-crystals where the material can be differentiated chemically, this consideration may be less important but it still applies to polymorphs of a given salt/cocrystal.

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Pharmaceutical Cocrystals: An Emerging Approach to Physical Property Enhancement

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Abstract

Pharmaceutical cocrystals are crystalline molecular complexes containing therapeutic molecules. They represent an emerging class of pharmaceutical materials offering the prospect of optimized physical properties. This article highlights important opportunities and challenges associated with the design and synthesis of pharmaceutical cocrystals. Cocrystallization is first placed into context with the more established approaches to physical property optimization of polymorphs, hydrates, and salt selection. A directed, intermolecular interaction-based approach to cocrystal design is described. The enhancement of specific physical properties, such as dissolution rate and physical stability, is illustrated by summarizing several recent reports. Synthetic approaches to cocrystallization are considered. In particular, the selectivity and screening-related opportunities afforded by solid-state grinding and solvent-drop grinding methods are discussed. Finally, an outlook on future developments summarizes the growth potential in this field, especially with regard to targeted, informatics-driven cocrystal screening approaches.

Keywords: biomedical, crystal growth, crystalline

Introduction

On average, about a decade of research and development is expended in the discovery and commercialization of a new pharmaceutical product. Initial R&D efforts center on the identification of a suitable molecular structure, physical form, and formulation. Whereas the molecular structure of the active pharmaceutical ingredient (API) of a drug substance is selected to optimize therapeutic properties, selecting the physical form of an API represents a strategic opportunity for optimizing such physical properties as solubility, dissolution rate, hygroscopicity, physical stability, and chemical stability.¹

Most APIs are dosed as solids, and most solid APIs exist in the crystalline form.

Frequently, however, the API does not crystallize on its own or it crystallizes into one or more crystal forms that possess undesirable physical properties. In either case, an alternative crystal form is typically sought. Various options include single-component and multiple-component modifications of an API, including polymorphs, salts, solvates, and hydrates. In addition to these established crystalline API modifications, pharmaceutical cocrystals, or crystalline molecular complexes involving an API, have recently attracted interest as an alternative approach.

This article outlines how pharmaceutical cocrystals offer an alternative approach to physical property optimization

during crystal form selection. Important design strategies for making cocrystals are described, along with some recent examples of using cocrystals to enhance specific physical properties. Cocrystal screening and synthesis are also covered, particularly using solid-state grinding and solvent-drop selective cocrystal synthesis.

Solid-State Modifications of APIs Polymorphs

A polymorph is "a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of that compound in the solid state."² Different polymorphs of a given compound each possess a unique set of physicochemical properties, and many, if not most, compounds exhibit polymorphism to some extent.^{1,3,4} Some compounds exist in more than ten crystal form modifications.⁵ At present, it is not generally possible to computationally predict the number of observable polymorphs of even the simplest molecules,⁶ and as a result, the use of high-throughput screening methods to search for new polymorphic forms has become an important tool in form screening.³

Hydrates and Solvates

Frequently during crystallization, solvent may be taken up and incorporated as part of the crystal structure. Most solvents, however, are biologically toxic; as a result, most solvate-containing crystals are avoided in the development of the solid form of an API.

An important exception is the subclass of API hydrates, which are well known in pharmaceutical products.^{7,8} It has been estimated that one-third of pharmaceutical molecules are capable of forming hydrates.^{9,10} As a result of process-induced stresses, such as changes in temperature, pressure, or relative humidity, hydrates often convert into anhydrous crystal forms. This conversion from hydrate to anhydrate can result in significant changes in physical properties and can present major issues, for example, during storage, where hydrate conversion can compromise dosage form appearance and integrity.

Pharmaceutical Salts

Salt formation is a common approach to modifying the properties of an API.¹¹⁻¹³ Salt formation is an acid-base reaction between the API and an acidic or basic substance. It is an attractive strategy, because most pharmaceutical compounds possess either acidic or basic functionality, and the widespread use of salt formation is evidenced by the large number of marketed crystalline salts of APIs.¹⁴

Pharmaceutical Cocrystals

A more recent approach to pharmaceutical physical property optimization is pharmaceutical cocrystal formation. A cocrystal may be thought of as a crystalline complex of two or more neutral molecular constituents bound together in the crystal lattice through noncovalent interactions, often including hydrogen bonding.

The application of cocrystallization to the pharmaceutical industry provides inherent benefits as compared with salt formation in at least two ways. The first is that, at least in theory, all types of molecules can form cocrystals, including weakly ionizable and non-ionizable APIs, which are traditionally considered to present a higher risk in terms of physical property optimization because they have either limited or no capacity for salt formation.

A second benefit is that, whereas for toxicological reasons only 12 or so acidic or basic counterions are explored in a typical API salt screen,^{12,13} there are many potential counter-molecules that may be used in cocrystal synthesis. (A counter-molecule may be defined as the species cocrystallized with the API.) The U.S. Food and Drug Administration manages several lists of substances that have precedence as food ingredients (e.g., the FDA's GRAS list, a list of substances "generally recognized as safe"), with the total amount of substances numbering in the thousands. Although the increased scope of cocrystals is a benefit in that it suggests a greater likelihood of achieving a desirable physical property profile for an API physical form, it also presents a challenge in terms of screening efforts, even with high-throughput screening.

To maximize efficiency in screening for cocrystals, therefore, improved rational cocrystal design and more efficient cocrystal screening protocols are needed.

Synthon Approach to Cocrystal Design

The formation of cocrystals has been studied for some time in academic research, and various important studies aimed at understanding cocrystal design have been published.

In early studies, Etter and co-workers proposed several "hydrogen-bond rules," including the observations that (1) all good proton donors and acceptors are used in hydrogen bonding, and (2) the best donor typically pairs with the best acceptor in a given crystal structure.¹⁶ The combined use of the hydrogen-bond rules with a geometric analysis (known as graph-set analysis¹⁷) assisted Etter and co-workers in implementing rational cocrystal design

in the synthesis of many new supramolecular structures.

Allen et al. demonstrated a quantification of the "robustness" of a certain class of intermolecular arrangements (commonly called motifs, or synthons) involving strong hydrogen-bonded bimolecular ring motifs. Their analysis involved examining trends within the Cambridge Structural Database (CSD), a searchable repository containing more than 300,000 small-molecule crystal structures.¹⁸ They assessed the robustness of a motif in terms of its "formation probability," that is, the observed frequency of motif formation among all structures containing the necessary functional group components. A higher formation probability suggested a greater utility in a cocrystal design scheme.

By relying on robust intermolecular interactions with demonstrated solid-state reproducibility, synthon-based cocrystal design has become increasingly important to the synthesis of new cocrystal materials. In the future, automated searches for formation probabilities pertaining to the molecular structure of an API of interest will be an important step toward rational pharmaceutical cocrystal design.

Pharmaceutical Cocrystals and Physical Property Enhancement

During the past few years, the focus on pharmaceutical cocrystals has increased significantly.¹⁹ In 2002, Oswald et al. demonstrated cocrystallization of the analgesic drug paracetamol (acetaminophen) with six different counter-molecules, each of which was capable of acting as a hydrogen-bond acceptor.²⁰ Shortly thereafter, Zaworotko and co-workers reported cocrystals of the APIs ibuprofen, flurbiprofen, and aspirin with several hydrogen-bond acceptors.²¹ These examples served as early proof that a series of cocrystals with common hydrogen-bonding features may be obtained with APIs. Aside from melting point data, however, these reports focused essentially on structural features without addressing the functional properties that these cocrystals might offer. Additionally, from an industrial standpoint, these were only model systems, in that the non-API components were, in most cases, not known to be safe for human ingestion.

In a subsequent paper, Zaworotko and co-workers reported on a series of cocrystals of the API carbamazepine, a drug used in the treatment of epilepsy, with a variety of different counter-molecules, including several that are biologically nontoxic, including acetic acid, nicotinamide (vitamin B₃), and the well-known sweetener saccharin.²² The report brought to light the

variety of possible counter-molecules that may form cocrystals with a single API.

Despite the increase in reports containing new pharmaceutical cocrystal structures, however, only a limited number of studies have directly addressed the realization of physical property modification. One report of a pharmaceutical cocrystal with enhanced dissolution properties involved cocrystals of several nontoxic C₄ (four-carbon) 1,4-dicarboxylic acids with itraconazole, an antifungal drug with very low aqueous solubility in its crystalline free base form.²³ The cocrystals reportedly resulted from a high-throughput crystal form screen of itraconazole, and the acids in the study were known to be biologically nontoxic at common pharmaceutical dosage levels.¹⁵ Single-crystal data were reported for one of the cocrystals, a 2:1 itraconazole:succinic acid cocrystal (Figure 1), where it was observed that the diacid spanned two itraconazole molecules via OH...N hydrogen bonds. All of the cocrystals demonstrated an enhanced dissolution profile as compared with itraconazole free base, and in some cases the dissolution profiles of the cocrystals approached that of amorphous itraconazole, which itself had been developed for the specific aim of enhancing the dissolution rate of the API.

The same study also emphasized the value of performing high-throughput cocrystal screening in addition to implementing rational design methodology. In the itraconazole:succinic acid cocrystal, the diacid formed a hydrogen bond with the five-membered triazole ring rather than with the most basic site on the drug molecule, the nitrogen of the six-membered piperazine (pK_a = 3.7). This example appeared to violate the best-donor-best-acceptor hydrogen-bond rule in preference for what may have been a geometric consideration: attempts to cocrystallize itraconazole with dicarboxylic acid chain lengths other than C₄, including malonic (C₃), glutaric (C₅), and adipic (C₆) acids, were reportedly unsuccessful. Until it becomes possible to confidently predict which counter-molecules will form cocrystals with a given API, high-throughput screening will continue to be of tremendous value to this research field.

A second demonstration of dissolution rate enhancement via cocrystallization involved three pharmaceutical cocrystals of the API fluoxetine, the active ingredient in the antidepressant drug Prozac.²⁴ This case was of particular interest from the standpoint of cocrystal design: the researchers formed cocrystals by combining a carboxylic acid with the hydrochloric acid (HCl) salt of fluoxetine, generating three novel cocrystals of salts. For example,

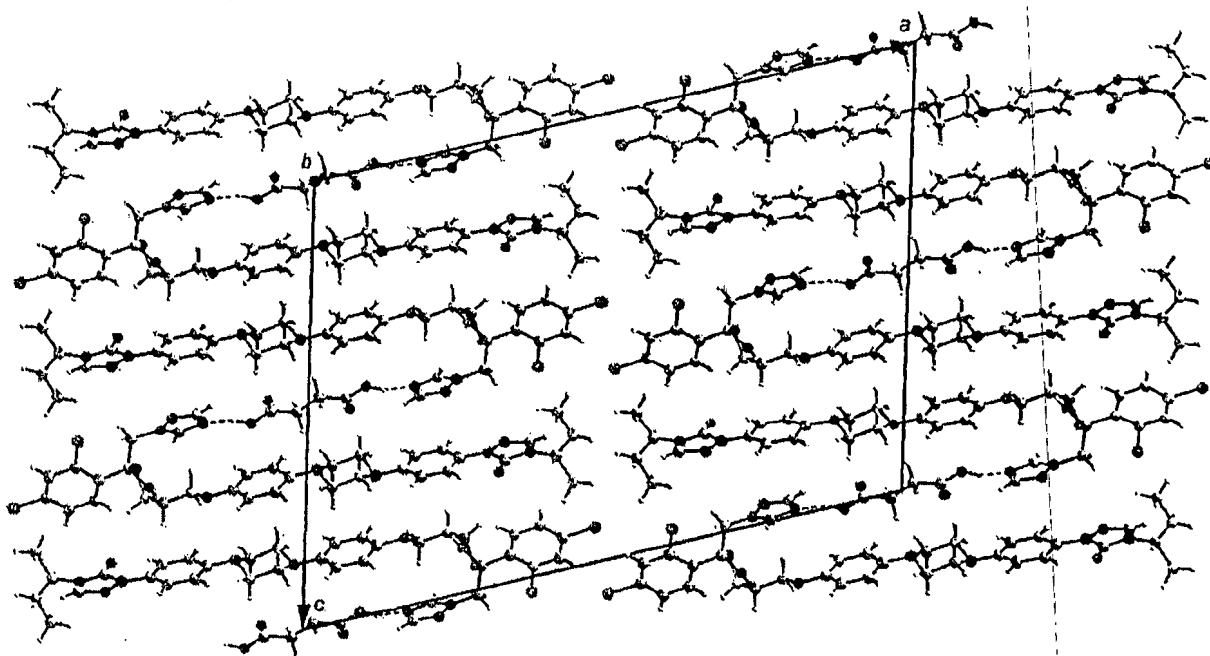


Figure 1. Crystal packing diagram and corresponding unit cell of the 2:1 itraconazole:succinic acid cocrystal.²³ Carbon atoms are large and gray, hydrogen atoms are small and white, nitrogen atoms are blue, oxygen atoms are red, and chlorine atoms are green.

fluoxetine:HCl salt was cocrystallized with succinic acid to form a succinic acid cocrystal of the fluoxetine:HCl salt, with a stoichiometry of 2:2:1 fluoxetine:chloride:succinic acid (see Structure 1). Significant differences in the measured dissolution rates of each of the three cocrystals were observed, such that individual cocrystals were found to exhibit rates above, below, and comparable with that of the crystalline HCl salt. In general, multiple-component cocrystals (and cocrystals of salts) with more than two components are evidence of the wide supramolecular diversity that may be achieved via cocrystal design.

The issue of physical stability enhancement via cocrystallization was

addressed using caffeine as a model API. Caffeine is known to exhibit solid-state physical instability as a function of relative humidity (RH); its stable anhydrous polymorph undergoes conversion to a crystalline hydrate upon exposure to high RH, and the hydrate loses water below a critical RH and reverts to the anhydrate. This form of physical instability limits the processing and storage conditions of an API in development. Caffeine also has a limited salt-forming capacity attributable to its weak basicity (its conjugate acid has a reported pK_a of 3.6), meaning that it is capable of forming salts only with strong acids. Only one pharmaceutically acceptable salt of caffeine had been reported in the CSD, a caffeine HCl salt that existed as a dihydrate.

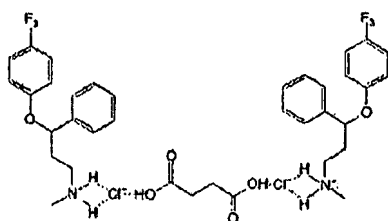
A cocrystallization study was initiated to obtain a series of cocrystals of caffeine that could be measured with regard to RH stability.²⁵ A strategy was devised whereby caffeine cocrystallization was attempted with several pharmaceutically acceptable dicarboxylic acids of various chain lengths. The strategy relied upon a caffeine-acid hydrogen-bond interaction that satisfied the hydrogen-bond rules, forming a motif that exhibited a good degree of robustness in the CSD.

Six caffeine:dicarboxylic acid cocrystals were reported, and the results of storing

cocrystal materials at several RH conditions were described. One cocrystal (the 2:1 caffeine:oxalic acid cocrystal, Figure 2), was physically stable at all RH conditions and all time points across the study. This cocrystal material was also fully stable upon slurrying in water. The stability of the caffeine:oxalic acid cocrystal is particularly remarkable given that both caffeine and oxalic acid, as pure materials, are known to convert to crystalline hydrates. The reason for this stability is currently being studied.

Supramolecular Synthesis via Solid-State Grinding

Solid-state grinding is the act of mixing, pressing, and crushing materials manually



Structure 1. Hydrogen-bond arrangement in the crystal structure of succinic acid cocrystal of fluoxetine:HCl salt; taken from crystal structure data.²⁴

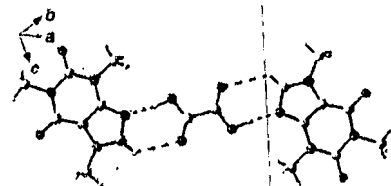


Figure 2. Hydrogen bonding in a 2:1 caffeine:oxalic acid cocrystal. Carbon atoms are large and gray, hydrogen atoms are small and white, nitrogen atoms are blue, and oxygen atoms are red.

with a mortar and pestle or mechanically in a mill. A common means of particle size reduction, solid-state grinding may also be performed on a mixture of materials to induce covalent or supramolecular reactivity. In the context of pharmaceutical cocrystals, solid-state grinding has emerged recently as a viable synthetic alternative to solution-based crystallization methods. In certain cases, pharmaceutical cocrystal synthesis by solid-state grinding offers enhanced selectivity as compared with that of solution crystallization. Moreover, the simplicity of the technique in revealing the cocrystallization potential between two molecular species suggests application in cocrystal screening efforts.

Caira and co-workers provided an early demonstration of the application of solid-state grinding to pharmaceutical cocrystal synthesis in a study of six cocrystals of the sulfa drug sulfadimidine with various carboxylic acids, including anthranilic acid (AA) and salicylic acid (SA).²⁶ Additionally, a remarkable preference was demonstrated for one particular cocrystal, the sulfadimidine:AA cocrystal. In a grinding competition experiment, a sulfadimidine:SA cocrystal, for which the crystal structure had been previously determined, was ground in the presence of AA. The result was a displacement of SA by AA as the cocrystal partner of sulfadimidine. Because of the common hydrogen-bonding pattern in both cocrystals, the authors based their explanation for the preference on the relative strengths of hydrogen bonding in the ingoing homomeric acid crystals. In extending these results to pharmaceutical processing considerations, it can be imagined that a grinding competition experiment such as the one just described might be used to assess the stability of a given pharmaceutical cocrystal material in the presence of excipients (i.e., substances other than the pharmacologically active drug in the final drug product) that may be encountered in the course of a formulation process.

With regard to the caffeine cocrystals described in the previous section, whereas single crystals were obtained by solution growth, it was reported that most cocrystals could also be prepared by grinding together the reactants in a ball mill. This finding was not unexpected: solid-state grinding has been repeatedly relied upon as a viable synthetic method for organic cocrystals. Solid-state grinding was often used by Elter and co-workers as a means of preparing cocrystal materials for the investigation of hydrogen-bond preferences. Furthermore, in a number of instances they reported that certain cocrystal modifications could be formed only by the

grinding method. These and other examples of this phenomenon were recently summarized.²⁷

The ability of solid-state grinding to reveal alternative cocrystal modifications would be particularly useful in pharmaceuticals, where unforeseen polymorphic transformations can bring disastrous consequences, including the withdrawal of a pharmaceutical product from the market.²⁸ For example, in a model system of cocrystals with caffeine and several monocarboxylic acids, solid-state grinding generated crystal forms which were initially inaccessible from solution. In experiments involving caffeine and trifluoroacetic acid, cocrystal material synthesis was initially found to be possible only via grinding. Two 1:1 polymorphs were identified and could be prepared separately depending upon the quantity of starting material in the grinding jars. The structure of each was solved from powder x-ray diffraction (PXRD) data. Subsequently, by using seeds obtained by grinding, cocrystal material of each polymorph was prepared by solution growth methods. This seeding method was used to obtain a single crystal for one of the structures, which confirmed the initial PXRD structure solution of that polymorph.²⁹

Enhanced Supramolecular Selectivity via Solvent-Drop Grinding

A modification to the solid-state grinding experiment has enabled enhanced supramolecular selectivity in certain cocrystal systems. Termed "solvent-drop" grinding, this method allows for stoichiometric and polymorphic selectivity in two model cocrystal systems.

Solvent-drop grinding involves the grinding of two materials together, as with solid-state grinding, but with the addition of a minor quantity of solvent (typically a few tenths of one equivalent of solvent per mole of starting material). The added solvent acts in what may be described as a catalytic role, in that the quantities employed are small and the solvent is not a component of the final cocrystal product.

The usefulness of solvent-drop grinding was first demonstrated in the context of cocrystallization rate enhancement in a system involving several cocrystals of nitrogenous bases with a cyclohexanetricarboxylic acid derivative, all of which were initially prepared by solution growth. It was found that some cocrystals could be readily prepared by solid-state grinding, whereas others exhibited only minor cocrystal content after grinding together starting materials for a significant time. For those that did not proceed to completion

upon solid-state grinding, it was found that solvent-drop grinding could be used to prepare an essentially phase-pure cocrystal material after significantly reduced periods of time.³⁰

Solvent-drop grinding was then found to enable selective polymorphic synthesis between two 1:1 caffeine:glutaric acid cocrystals (Forms I and II).³¹ The two polymorphs, which shared an identical sheet-like hydrogen-bonding arrangement and differed primarily in terms of the stacking of sheets, were first found to precipitate concomitantly from solution. In an effort to prepare each polymorphic modification separately, grinding was explored as a method of cocrystal synthesis. It was found that solid-state grinding of caffeine and glutaric acid produced predominantly Form I and that solvent-drop grinding with polar solvents (cyclohexane, hexane, and heptane) also produced Form I in the absence of Form II. Alternatively, phase-pure Form II could be prepared by the grinding of starting materials in the presence of more polar solvents (acetonitrile, chloroform, and water). A possible factor that may have had a role in this observed selectivity was the observation of a potential nonpolar cleavage plane in the Form I polymorph. Examples have also been reported of stoichiometric selectivity via solvent-drop grinding.³²

In addition to the ability of solvent-drop grinding to provide for polymorphic and stoichiometric selectivity in cocrystallization, the technique has also been demonstrated as a way of interconverting crystal forms of polymorphic organic materials, such as succinic acid and anthranilic acid.³² In the case of succinic acid, grinding of the stable polymorph in the presence of nonpolar solvents was found to result in significant quantities of the metastable polymorph, hitherto known to crystallize only at high temperatures. AA, a trimorphic system, underwent interconversions between the three different polymorphs depending upon the solvent that was added in minor quantities to the grinding experiment. A schematic depicting the interconversions among AA polymorphs is provided as Figure 3.

Solvent-drop grinding has also found application with regard to crystalline salt synthesis with pharmaceuticals.³³ Salt screening is an important aspect of physical property optimization, as well as intellectual property protection, for many API candidates. Much effort, increasingly using high-throughput robotics, is expended in revealing all potential salts (and polymorphs of salts) to ensure that the salt selection is made from the widest knowledge of potential candidates.³

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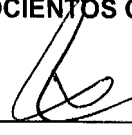
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