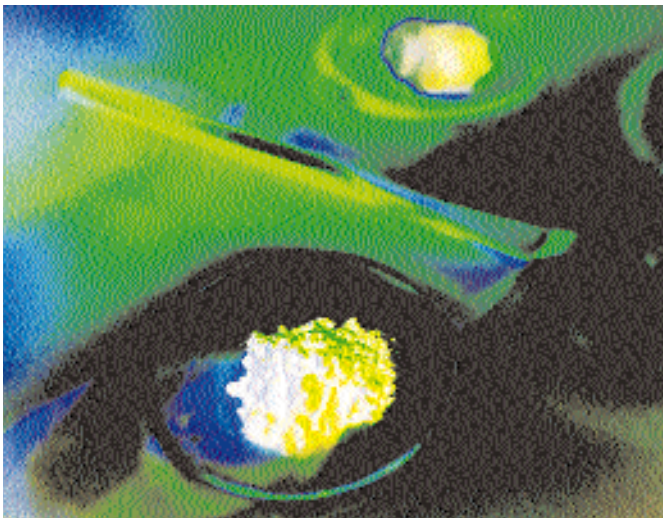
# X-ray Diffraction III: Pharmaceutical Applications

## of X-ray Powder Diffraction



**Harry G. Brittain**
Center for Pharmaceutical Physics

**X-ray powder diffraction is the predominant tool for the study of polycrystalline materials and is eminently suited for the routine characterization of pharmaceutical solids.**

Although the solving of a crystal provides the greatest understanding of its crystallographic structure, the necessity of obtaining suitable single crystals and the degree of complexity associated with the data analysis precludes this technique from being used on a routine basis for batch characterization. In fact, most drug substances are obtained as microcrystalline powders that do not contain crystallographically adequate crystals. During the most common evaluation of drug substances it is usually sufficient only to establish the polymorphic identity of the solid and to verify that the isolated compound is indeed of the desired structure (1).

Parts I and II in a series of articles covering x-ray diffraction examined the phenomenon of the diffraction of x-rays by crystalline solids, briefly discussed the lattice theory of crystal structure, and outlined the use of single-crystal diffraction for the study of polymorphism and solvatomorphism (2, 3). This article concludes the series by presenting a brief exposition of x-ray powder diffraction (XRPD).

The XRPD technique is exceedingly important in pharmaceutical physics because it is the easiest and fastest method to obtain fundamental information about the structure of a crystalline substance. Structural analysis by single-crystal x-ray diffraction provides the largest amount of information but is significantly harder to obtain and requires a suitable crystal (4, 5). Because the majority of drug substances are obtained as crystalline powders, researchers often use the powder pattern of these substances as a readily obtainable fingerprint to determine structural type. In fact, it is only by pure coincidence that two compounds might form crystals for which the ensemble of molecular planes happen to be identical in all space. One such example is the trihydrate phases of ampicillin and amoxicillin (6), but such instances are uncommon.

Bragg explained the diffraction of x-rays by crystals using a model in which the atoms of a crystal are regularly arranged in space and can be regarded as lying in parallel sheets separated by a definite and defined distance $d$ (7). He then showed that scattering centers arranged in a plane act like a mirror to x-rays incident on them, so that constructive interference occurs for the direction of specular reflection. Within a given family of planes, defined by a Miller index of ($hkl$), each plane produces a specular reflectance of the incident beam. If the incident x-rays are monochromatic having wavelength $\lambda$, then for an arbitrary glancing angle of $\theta$, the reflections from successive planes are out of phase with one another. This yields destructive interference in the scattered beams. However, by varying $\theta$, a set of values for $\theta$ can be found so that the path difference between x-rays reflected by successive planes will be an integral number $n$ of wavelengths, and constructive interference occurs. One ultimately obtains the following equation, known as Bragg's law, that explains the phenomenon:

$$2d\sin\theta = n\lambda \qquad [1]$$

**Table I.** XRPD identity test criteria for benzoic acid.

| *d*-spacing (Å) | Lower Limit of Scattering Angle (degrees 2-θ) | Reference Scattering Angle (degrees 2-θ) | Upper Limit of Scattering Angle (degrees 2-θ) | Lower Limit of Relative Intensity ($I/I_0$) | Reference Relative Intensity ($I/I_0$) | Upper Limit of Relative Intensity ($I/I_0$) |
|---|---|---|---|---|---|---|
| 5.1482 | 17.00 | 17.20 | 17.40 | 100 | 100 | 100 |
| 10.8662 | 7.93 | 8.13 | 8.33 | 76 | 95 | 114 |
| 5.4368 | 16.09 | 16.29 | 16.49 | 44.8 | 56 | 67.2 |
| 3.7334 | 23.61 | 23.81 | 24.01 | 21.6 | 27 | 32.4 |
| 3.4437 | 25.65 | 25.85 | 26.05 | 20.0 | 25 | 30.0 |
| 4.6452 | 18.89 | 19.09 | 19.29 | 16.8 | 21 | 25.2 |
| 3.2099 | 27.57 | 27.77 | 27.97 | 13.6 | 17 | 20.4 |
| 2.9636 | 29.93 | 30.13 | 30.33 | 12.8 | 16 | 19.2 |
| 2.5723 | 34.65 | 34.85 | 35.05 | 9.6 | 12 | 14.4 |
| 3.6260 | 24.33 | 24.53 | 24.73 | 7.2 | 9 | 10.8 |

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

To measure a powder pattern, one would prepare a randomly oriented powdered sample to expose all possible planes of a crystalline powder. One can measure the scattering angle, θ, for each family of crystal planes by slowly rotating the sample and measuring the angle of diffracted x-rays with respect to the angle of the incident beam. Alternatively, the angle between sample and source can be kept fixed while the detector is moved to determine the angles of the scattered radiation. Knowing the wavelength of the incident beam, one can calculate the spacing between the planes (identified as the d-spacings) using Bragg's law.

Typical applications of x-ray powder diffraction methodology include the evaluation of polymorphism and solvatomorphism, the study of phase transitions, and evaluation of degrees of crystallinity. More recently, researchers have made advances in the use of powder diffraction as a means to obtain solved crystal structures. A very useful complement to ordinary XRPD is variable-temperature x-ray diffraction. In this method, the sample is contained on a stage that can be heated to any desired temperature. The method is extremely useful for the study of thermally induced phenomena and can be a vital complement to thermal methods of analysis.

## CONVENTIONAL XRPD: PHASE IDENTITY OF MATERIALS

The 1995 *USP 23/NF 18* General Chapter on x-ray diffraction states that identity is established if the scattering angles of the 10 strongest reflections obtained for an analyte agree to within ±0.20 degrees 2-θ with that of the reference material and if the relative intensities of these reflections do not vary by more than 20% (8). Applying these compendial acceptance criteria to the published pattern of benzoic acid leads to the XRPD specifications shown in Table I (9). For a given benzoic acid sample to pass an XRPD identity test, its scattering peaks must fall within the boundaries defined in the table. The intensity rule was effectively removed in *USP 24/NF 19,* which states that relative intensities may vary considerably from that of the reference standard (10).

## CONVENTIONAL XRPD: DEGREE OF CRYSTALLINITY

When reference samples of the pure amorphous and the pure crystalline phases of a substance are available, calibration samples of known degrees of crystallinity can be prepared by the mixing of these. By establishing a calibration curve of the XRPD response versus the degree of crystallinity, unknown samples can be evaluated. For instance, Otsuka et al. reported a study involving partially crystalline indomethacin (11). They obtained a commercially available crystal form and generated the amorphous phase by quench-cooling the melted substance. They then prepared calibration mixtures as various blends of the 100% crystalline and 0% crystalline materials and obtained acceptable linearity ($r^2$ = 0.988) in the calibration curve of XRPD intensity (based on three peaks) versus actual crystallinity.

## CONVENTIONAL XRPD: POLYMORPHISM AND SOLVATOMORPHISM

One of the most important uses of XRPD in pharmaceuticals is its application as the primary determinant of polymorphic or solvatomorphic identity. Because polymorphism and solvatomorphism are the results of purely crystallographic phenomena, x-ray diffraction represents the essential method of determination. Because of its ease of data acquisition, XRPD

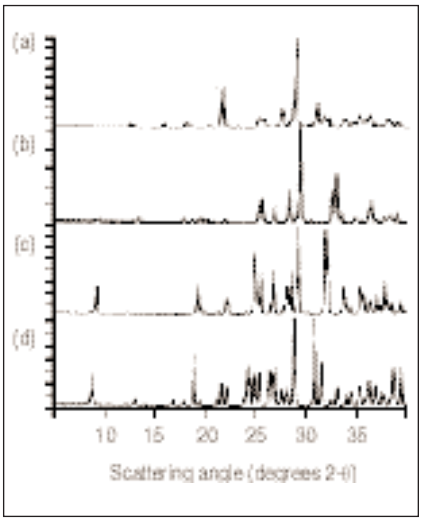



**Figure 1.** X-ray powder diffraction patterns of (a) sodium, (b) potassium, (c) rubidium, and (d) cesium Group IA cation adducts formed with 5-nitrobarbituric (dilituric) acid. The data are adapted from reference 12.

**Table II.** Crystallographic parameters of roxifiban deduced using synchrotron XRPD and electron diffraction (15).

| | Form I (Triclinic) | Form II (Monoclinic) |
|---|---|---|
| Space group | P1 | $P2_1$ |
| Unit cell occupancy | Z = 2 | Z = 4 |
| Unit cell lengths | $a$ = 5.0235 Å | $a$ = 4.992 Å |
| | $b$ = 28.075 Å | $b$ = 54.770 Å |
| | $c$ = 9.2954 Å | $c$ = 9.372 Å |
| Unit cell angles | α = 98.53° | α = 90° |
| | β = 98.50° | β = 99.15° |
| | γ = 92.24° | γ = 90° |

is particularly useful as a screening technique for batch characterization, and the criteria already described for phase identification can serve to differentiate polymorphs or solvatomorphs. It is prudent, however, to verify the results of an XRPD study with a confirmatory technique such as polarizing light microscopy, differential scanning calorimetry, solid-state vibrational spectroscopy, or solid-state nuclear magnetic resonance (12).

To illustrate the use of XRPD as a means of phase or compound identification, consider the adducts formed by Group IA cations with 5-nitrobarbituric (dilituric) acid (13). This system was studied because dilituric acid had been used extensively as a chemical microscopic reagent for the qualitative identification of Group IA and Group IIA cations, but the origin for the myriad observed crystal morphologies had not been satisfactorily explained. Figure 1 shows the XRPD patterns of most of the Group IA adducts and the unique crystal structure exhibited by each of the adduct species. This finding indicates that the differing cation–dilituric radius ratio values result in differing packing arrangements in the solids. These various packing arrangements yield the range of observed crystal structures, and this polymorphism becomes evident in new crystal morphologies. Other adduct species were observed to form various hydrate structures, ranging from sesquihydrates to trihydrates. These other structures also yielded various crystal morphologies owing to the existence of various hydrate species, which also would contain structural variations as a result of cation–dilituric packing patterns.

## CONVENTIONAL XRPD: PHASE COMPOSITION OF MIXTURES

The antiulcer drug ranitidine hydrochloride is known to crystallize in two anhydrous polymorphs. Figure 2 shows the XRPD patterns of the two significantly different forms (14). For various reasons, most researchers are extremely interested in determining the quantity of Form II in a bulk Form I sample, and it is clear from the figure that the scattering peaks around 20 and 23.5 degrees 2-θ would be particularly useful for this purpose. Rarely does one encounter this extremely favorable situation in which the most intense scattering peaks of the analyte form are observed at scattering angles where the host matrix happens not to yield diffraction. Clearly, this serendipitous situation would create fairly low limits of detection for Form I in the presence of Form II (15). On the other hand, if there were a reason to determine the level of Form I in a bulk Form II sample, then the scattering peaks around 9 and 25 degrees 2-θ would suffice.

## SYNCHROTRON XRPD: CRYSTALLOGRAPHIC PROPERTIES

A given sample consists of crystallographically pure material if that sample's powder pattern can be successfully indexed (for example, a Miller index can be assigned to each and every observed scattering peak). This process usually is extremely difficult to complete for low-symmetry organic compounds, especially when the XRPD data are obtained using ordinary instrumentation. When using synchrotron radiation as the source, however, researchers can obtain powder patterns at high resolution (<0.03 degrees 2-θ). These high-quality patterns can be processed using indexing programs to deduce unit cell dimensions for the sample being studied. The data can be used as input for molecular modeling programs and refined with Rietveld analysis to deduce crystal structures from the powder data.

The two polymorphs of roxifiban are difficult to distinguish by conventional XRPD and polarizing light microscopy, but Li et al. obtained structural information using a combination of synchrotron XRPD and electron diffraction techniques (16). Table II shows a summary of the reported crystallographic information. The researchers concluded that even when crystallographic-grade crystals cannot be grown to permit single-crystal analysis, one can select the proper experimental methodology to deduce structural information.

## VARIABLE-TEMPERATURE XRPD: STRUCTURAL INTERPRETATION FOR THERMAL TRANSITIONS

The assignment of the origin of thermal events detected during the conduct of differential thermal analysis or differential scanning calorimetry (DSC) is not always straightforward, and the use of supplementary technology is frequently desirable. By performing XRPD studies on a heatable stage, one can bring the system to positions where a DSC thermogram indicates the existence of an interesting point of thermal equilibrium. For instance, after the dehydration of a hydrate phase, one may obtain either a crystalline anhydrate phase or an amorphous phase. The XRPD pattern of a dehydrate hydrate will clearly indicate the difference. In addition, should one encounter an equivalence in powder patterns between the hydrate phase and its dehydrated form, this would indicate the existence of channel-type water (as opposed to genuine lattice water) (17).

The commercially available form of aspartame is hemihydrate Form II, which

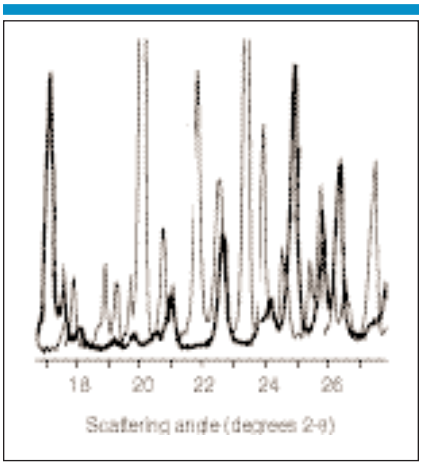



**Figure 2.** X-ray powder diffraction patterns of ranitidine hydrochloride. Form I (thick trace) and Form II (thin trace) (13).

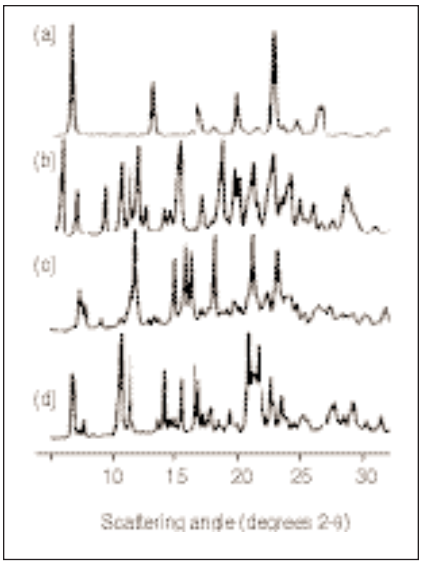



**Figure 3.** X-ray powder diffraction patterns of aspartame (a) dioxopiperazine degradent, (b) anhydrate, (c) hemihydrate, and (d) 2.5 hydrate. The data are adapted from Reference 13.

transforms into hemihydrate Form I when milled. A 2.5-hydrate species also is known (18). Researchers have used XRPD to study the desolvation and ultimate decomposition of the various hydrates. When heated to 150 °C, both hemihydrate forms dehydrate into the same anhydrous phase, which then cyclizes to 3-(carboxymethyl)-6-benzyl-2, 5-dioxopiperazine if heated to 200 °C. The 2.5 hydrate can dehydrate to hemihydrate Form II when heated to 70 °C, which can undergo the same decomposition sequence as directly crystallized hemihydrate Form II. Figure 3 shows various XRPD patterns obtained during the heating sequence of the 2.5 hydrate.

## VARIABLE-TEMPERATURE XRPD: ACCELERATED STABILITY STUDIES

The physical quality of a drug substance during the conduct of stability studies must be well established, and XRPD is certainly capable of being validated to the status of a stability-indicating assay. The crystallographic stability of a drug substance can be studied using XRPD as part of the protocol, which is especially important when one chooses either a metastable or an amorphous form of the drug substance for development. One may conduct such work either on samples that have been stored at various conditions and pulled at designated time points or on substances that are maintained isothermally and the XRPD periodically measured.

Yonemochi et al. prepared amorphous clarithromycin by grind and spray-drying processes and used XRPD to follow changes in crystallinity on exposure to elevated temperature and relative humidity (18). Exposure of either substance to a 40 °C and 82% relative humidity environment for seven days led to formation of the crystalline product, but the spray-dried material yielded more crystalline form than did the ground material. This finding, when supported with thermal analysis studies, led to the conclusion that the amorphous substances produced by the different processing methods were not equivalent.

## SUMMARY

XRPD represents the methodology of choice for the crystallographic characterization of drug substances produced on a routine, batch-type basis. Properly prepared samples yield powder patterns that contain a scattering peak for each crystal plane or face and therefore constitute an identification test for a given crystalline phase. When the data are of suitable quality, XRPD can be used to deduce details of the unit cell and the crystal structure. With the generation of appropriate calibration data, XRPD can be used to deduce the degree of crystallinity in a given sample and the composition of a physically heterogeneous mixture. Because polymorphism and solvatomorphism are crystallographic occurrences, XRPD will always be the primary determinant of the existence of such phenomena. Finally, variable-temperature XRPD is a valuable tool to understand thermally induced reactions and to characterize materials during stability studies.

## REFERENCES

(1) J. Anwar, "Pharmaceuticals: Design and Development of Drug Delivery Systems," in *Industrial Applications of X-ray Diffraction*, F.H. Chung and D.K. Smith, Eds. (Marcel Dekker, New York, 2000), pp. 539–553.
(2) H.G. Brittain, *Spectroscopy* **15**(6), 44–49 (2000).
(3) H.G. Brittain, *Spectroscopy* **15**(7), 34–39 (2000).
(4) M.M. Woolfson, *X-ray Crystallography* (Cambridge University Press, Cambridge, 1970).
(5) G.H. Stout and L.H. Jensen, *X-ray Structure Determination: A Practical Guide* (Macmillan Co., New York, 1968).
(6) M.O. Boles, R.J. Girven, and P.A.C. Gane, "The Structure of Amoxicillin Trihydrate and a Comparison with the Structures of Ampicillin," *Acta Cryst.* B34, 461 (1978).
(7) W.H. Bragg and W.L. Bragg, *X-rays and Crystal Structure* (G. Bell & Sons, London, 1918).
(8) "X-ray Diffraction," General Chapter (941), *USP 23/NF 18* (The United States Pharmacopoeial Convention, Rockville, MD, 1995), pp. 1843–1844.
(9) G. Indrayanto et al., "Benzoic Acid," in *Analytical Profiles of Drug Substances and Excipients, Volume 26*, H.G. Brittain, Ed. (Academic Press, San Diego, CA, 1999), pp. 10–11.
(10) "X-ray Diffraction," General Chapter (941), *USP 24/ NF 19* (United States Pharmacopoeial Convention, Rockville, MD, 2000), pp. 2005–2007.
(11) M. Otsuka, F. Kato, and Y. Matsuda, "Comparative Evaluation of the Degree of Indomethacin Crystallinity by Chemoinfometrical Fourier-Transformed Near-Infrared Spectroscopy and Conventional Powder X-ray Diffractometry," *AAPS-PharmSci.* **2**, Issue 1, Paper 9 (2000).
(12) S. Byrn et al., "Pharmaceutical Solids: A Strategic Approach to Regulatory Considerations," *Pharm. Res.* **12**, 945–954 (1995).
(13) H.G. Brittain, "Foundations of Chemical Microscopy I: Solid-State Characterization of 5-Nitrobarbituric Acid (Dilituric Acid) and its Complexes with Group IA and Group IIA Cations," *J. Pharm. Biomed. Anal.* **15**, 1143–1155 (1997).
(14) H.G. Brittain, unpublished results.
(15) S. Agatonovic-Kustrin et al., "Powder Diffractometric Assay of Two Polymorphic Forms of Ranitidine Hydrochloride," *Int. J. Pharm.* **184**, 107–114 (1999).
(16) Z.G. Li et al., "Polymorph Determination for the GP IIb/IIIa Antagonist, Roxifiban, Using a Combination of Electron Diffraction and Synchrotron X-ray Powder Diffraction Techniques," *J. Pharm. Sci.* **88**, 297–301 (1999).
(17) G.A. Stephenson et al., "Formation of Isomorphic Desolvates: Creating a Molecular Vacuum," *J. Pharm. Sci.* **87**, 536–542 (1999).
(18) E. Yonemochi et al., "Physicochemical Properties of Amorphous Clarithromycin Obtained by Grinding and Spray Drying," *Eur. J. Pharm. Sci.* **7**, 331–338 (1999).

**Harry G. Brittain** *is director of the Center for Pharmaceutical Physics (an affiliate of Magellan Laboratories), 10 Charles Road, Milford, NJ 08848. He can be contacted by phone at (908) 996-3509, by fax at (908) 996-3560, or by e-mail at hbrittain@earthlink.net. This article is the third installment of a series also appearing in Brittain's "Pharmaceutical Physics" column in* Pharmaceutical Technology *magazine, a sister publication of* Spectroscopy. ◆