Polymorph Determination for the GP IIb/IIIa Antagonist, Roxifiban, Using a Combination of Electron Diffraction and Synchrotron X-ray Powder Diffraction Techniques

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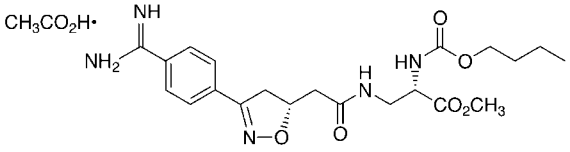
Abstract □ Unit cell parameters of two polymorphs of roxifiban have been determined by a combination of transmission electron microscopy (TEM) single-crystal and synchrotron X-ray powder diffraction techniques. While it was difficult to differentiate the two forms by their standard X-ray diffraction patterns, the high-resolution synchrotron patterns clearly showed striking differences. Unit cells for the two forms required the use of cell parameters derived from TEM diffraction patterns. The two unit cells are, not surprisingly, very similar except for a doubling of one of the axes for form II. The combined use of TEM and synchrotron patterns appears to be a good general approach for characterizing complex (low-symmetry, large unit cell) polymorphs.

Introduction

The term polymorph refers to substances that have the same chemical formula but different crystal structures. Polymorphism has played an important role in drug development, production, and litigation. Synthesized and crystallized under different conditions, polymorphs may have different physical and chemical properties as well as bioavailabilities. Steroids, sulfonamides, and barbiturates are some well-known examples[1] of compounds that have two or more polymorphs. Recently, the polymorphic behavior of Abbott-79175 was detailed.[2] The conventional techniques used to identify polymorphs are X-ray diffraction, optical microscopy, DSC, IR, and solid-state NMR. However, these techniques sometimes fail to differentiate definitively between two structurally similar polymorphs, so more advanced techniques are required. The advanced techniques were applied to roxifiban:



The GP IIb/IIIa receptor antagonist, roxifiban, is a very promising cardiovascular drug discovered recently at DuPont Pharmaceuticals.[3,4] Extensive human clinical trials are presently in progress. It was suspected at one point that different crystalline phases, labeled form I and form II, were being obtained when the synthesis/crystallization conditions were varied. Bulk and tapped densities, for example, were found to vary from 0.23 and 0.37 g/mL for form I to 0.07 and 0.19 g/mL for form II, respectively. These differences were of some concern given the low drug load in the solid dosage form.

Conventional X-ray single-crystal diffraction and polarized microscopy, two traditional methods employed to characterize the physical forms of drug compounds in development, were of no value in distinguishing between the two forms because of the relatively small crystallite sizes of the materials. Even powder diffraction patterns of forms I and II of roxifiban showed few differences when collected on a commercial diffractometer; although there is some intensity variation among peaks in general, there were only 1−2 additional peaks that were truly unique to form II. These differences in the two patterns could easily be explained away either as an effect of preferred orientation (since the crystals have a needle habit) or by the presence of impurity phases.

Unit cell parameters and atomic coordinates of crystalline polymorphs can routinely be determined by single-crystal X-ray diffraction techniques if suitably large crystals are available. However, many organic compounds tend to grow needle and platelike crystals that never achieve a large enough volume for single-crystal studies. Such was the case with forms I and II of roxifiban: diffraction patterns from a few small "single-looking" crystallites produced just enough spots to clearly show that these crystals were generally twinned or agglomerated. Because the single-crystal technique was not available in this case, both TEM and synchrotron powder diffraction techniques were employed in the hope of at least determining the unit cells for the two forms. If all of the peaks in the individual powder patterns of forms I and II can be accounted for by appropriate indexing of the peaks based on their unit cell parameters, then one can assume with high probability (a) that the two forms do indeed exist and, therefore, can be distinguished and (b) that the peaks are not from contaminant phases.

While it is possible to obtain unit cell information from powder X-ray diffraction data by "trial-and-error" techniques either manually or through the use of automated indexing software, chances for successful determination are less likely if the unit cell has low symmetry and a large volume. Unit cells are easily determined by single-crystal techniques because the diffraction spots are spread out in a three-dimensional pattern. Because the powder spectrum is a projection of these spots into a one-dimensional set of peaks, wrong unit cells are often determined when low-resolution commercial powder patterns are used as input.

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High-resolution powder diffractometers at synchrotron sources produce patterns that are more easily indexed, but complicated patterns of the type produced by many organic crystals are still difficult to analyze. In electron diffraction, two-dimensional patterns can be obtained from crystals which are very small (submicron or less in size). This is a big advantage in determining some of the unit cell parameters, but generally it is difficult (crystals are generally plates or needles) to obtain all three of the necessary projections to determine all the parameters. Another disadvantage is that organic materials are usually beam sensitive and, for this reason, electron diffraction has not been extensively used on pharmaceuticals. Recently, however, the development of very sensitive CCD detectors has allowed electron diffraction patterns of beam-sensitive materials to be recorded in a few seconds using very low electron currents. In this report, we describe the utility of our newly developed "hybrid" characterization technique[5,6] to pharmaceuticals, coupling the analysis of the synchrotron powder data with information obtained from TEM diffraction patterns to characterize the unit cell parameters of the two forms of roxifiban.

Experimental Section

High-resolution, X-ray powder diffraction patterns for various samples of roxifiban were obtained at the Advanced Photon Source, a DOE User Facility located at Argonne National Laboratory, Chicago, IL. The samples were initially mounted in capillaries although flat-plate samples were later used to obtain patterns with increased Bragg intensities. Patterns were collected on a Huber diffractometer at beamline DND-5BMB. A Si(111) double-crystal monochromator, a Si(111) analyzer, and slits on the order of 1 × 8 mm were used in conjunction with a scintillation counter to achieve the highest possible resolution and signal/noise ratio. Peaks in the patterns were deconvolved using GPLSFT, and indexing was attempted using the program TREOR. GSAS was used to test possible unit cells.

A JEM-2000EX (at 200 kV accelerated voltage) microscope, equipped with a Gatan 1024 × 1024 CCD camera, was used to characterize these materials. The samples were directly deposited on conventional TEM grids. The TEM technique has the primary advantage of being able to examine individual crystallites, and thus it can circumvent the problems of contaminant phases. This technique can exploit the special geometric shapes of these crystallites, many of which have plate and fiberlike habits. In particular, unit cell information perpendicular to the plate direction can be obtained: a typical diffraction pattern might contain the *h*0*l* zone, for example, from which the *a**, *c**, and β* parameters can be obtained.

While these two techniques were individually valuable for partial definitions of the unit cell, neither give data from which the complete unit cell could readily be determined. The determination of roxifiban polymorphs required a combination of transmission electron microscopy (TEM) and synchrotron X-ray diffraction capabilities.

Results

Electron Microscopy (EM)—Figure 1 shows scanning electron microscopy images comparing the morphologies of forms I and II. Both forms I and II contain very small crystals which prevent the use of conventional single crystal X-ray techniques. It is clear that the crystals of form II are more fiberlike. Figure 2 is a low magnification TEM view of form I and II crystals, confirming the SEM results. Figure 3 shows a higher magnification image of a single Form II crystal and its diffraction pattern. TEM results indicate that *d*(100), *d*(001), and β* of both forms I and II are, respectively, around 5.0 Å, 9.2 Å, and 81°.

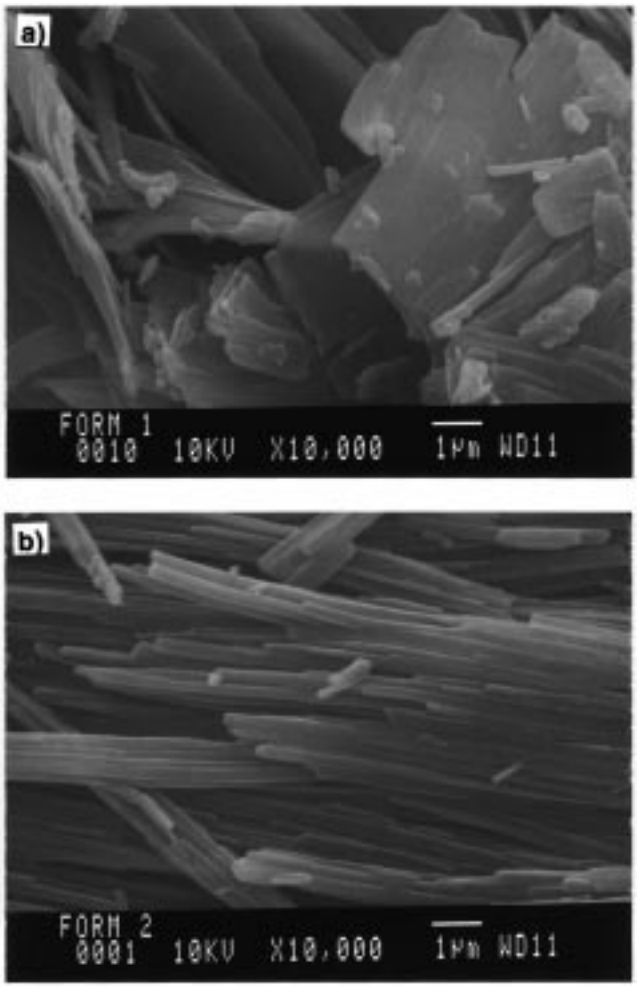

Figure 1—Scanning electron microscopy images of forms I and II. The crystals of form II are seen to be much more fiberlike.

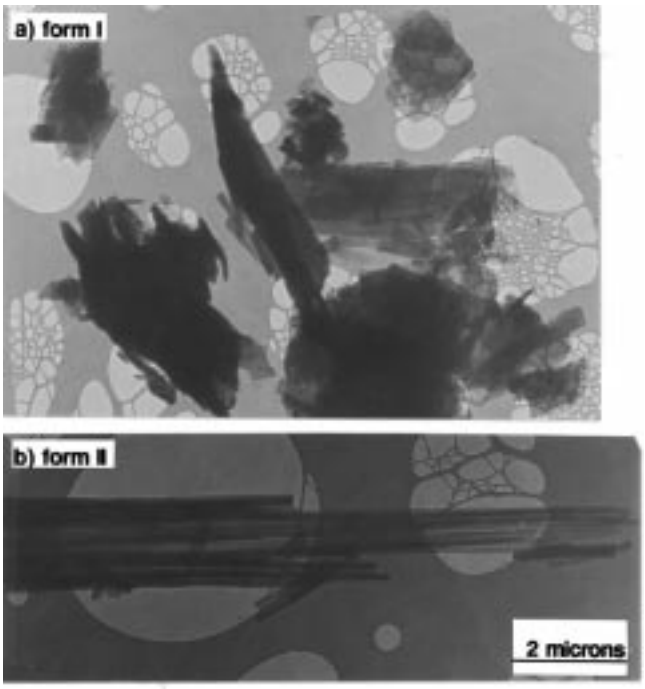

Figure 2—Low magnification TEM images of (a) form I and (b) form II crystals.

Synchrotron X-ray Powder Diffraction (S-XRPD)—*Transmission Mode*—It was revealed that the S-XRPD

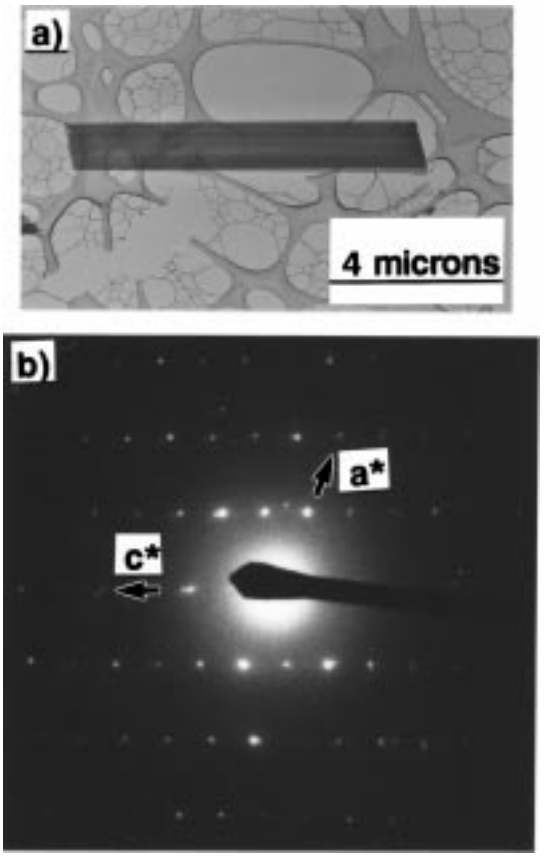

Figure 3—(a) TEM image and (b) diffraction pattern of a single form II crystal.

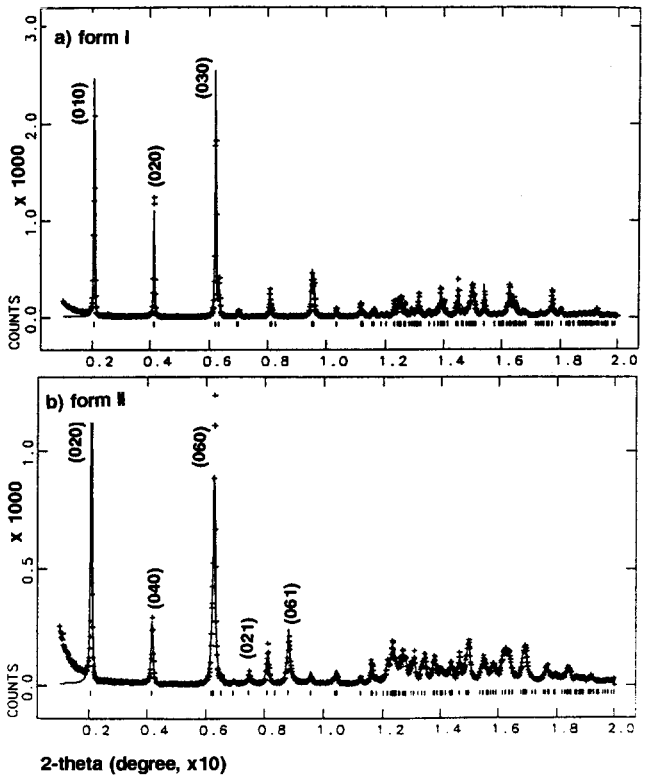

Figure 4—Synchrotron X-ray diffraction patterns (wavelength of 1.00006 Å) of (a) form I and (b) form II. In both cases, the "+" marks represent the observed data points; the solid line is the result of a LeBail fit using the unit cell parameters. The "tick" marks are the locations of the expected reflections. The two extra peaks in the low-angle region of the form II pattern which clearly differentiate it from the pattern of form I are labeled as the (021) and (061) reflections. It can clearly be seen that the match of the derived unit cells and the experimental data points is excellent.

diffraction pattern of form I and form II run with a 1.5 mm capillary were indeed different with two extra diffraction peaks in the lower angle range. Since the large capillary samples should be relatively free of preferred orientation, it was assumed at this point that the major differences in the patterns were a clear indication that the extra peaks in form II were not simply due to preferred orientation effects.

Reflection Mode—Because the intensities of the diffraction lines using the capillary mode were relatively weak, we recollected the patterns in reflection mode using large and deep flat-plate sample holders on samples that were carefully ground. Figure 4 shows the resulting S-XRPD patterns for forms I and II and points out the two extra peaks in the low-angle region of form II which (vide infra) distinguish it from form I. Although we had expected serious problems from preferred orientation effects, the relative intensities of the peaks in the reflection mode were, in fact, very similar to those measured in the capillary mode (without grinding the samples, the relative intensities in reflection mode were very different and strong, preferred orientation effects were very obvious).

Unit Cell of Form I—An attempt was made to determine the unit cell of form I since the peaks were reasonably sharp and the phase appeared pure. TEM work had suggested that the cell was probably of low symmetry, either monoclinic or triclinic, with two of the three cell axes in the neighborhood of 5.0 and 9.2 Å with an interaxial angle of 81°. Synchrotron patterns had shown that the third axis was very long, ca. 27−28 Å. Given the number of atoms in the molecule, one can estimate that the volume per molecule is in the vicinity of 648 Å^3 which would be compatible with two molecules per cell. Indexing was attempted first in the triclinic study giving several hits with one in particular that had dimensions of 6 × 9 × 28 Å. Placing the trial cell into the program RMANDEX showed a nearly perfect fit to all of the peaks. The cell parameters were then refined with CELLREF, and ultimately these numbers were used in a LeBail fit routine in GSAS. Figure 4a shows the quality of the fit for the full pattern. The refined triclinic ($Z = 2$, with space group P1 because the compound is stereochemically pure) unit cell parameters are given below:

	a	*b*	*c*	α	*β*	*γ*	*V*
value:	5.0235	28.075	9.2954	98.533	98.498	92.244	1279.7
σ:	0.0005	0.002	0.0009	0.006	0.009	0.008	0.2

Unit Cell of Form II—Several lines in the low-angle regions of the pattern are unique to the form II pattern, and if their origin could be established, then it might be possible to determine its unit cell. The form II pattern was displayed in RMANDEX[7] using the same unit cell established for form I as a starting point because TEM results indicated that a^*, c^*, and β^* values of forms I and II were very similar. It became immediately clear that the unique peaks in the pattern could not be indexed unless one of the cell edges was doubled. Doubling the volume requires that four molecules must reside in the cell, and, in that case, the cell would likely be monoclinic with space group $P2_1$. The long axis, ca. 28 Å, was the obvious choice for *b* (the unique axis of the monoclinic cell) because the TEM diffraction pattern suggested that 5−9 Å projection still had the 81° angle. Fixing the α and *γ* angles to 90° and adjusting the *a* and *c* axes as well as the *β* angle soon gave a cell which could account for the unique lines in the form II pattern. This cell was then refined using the LeBail fit

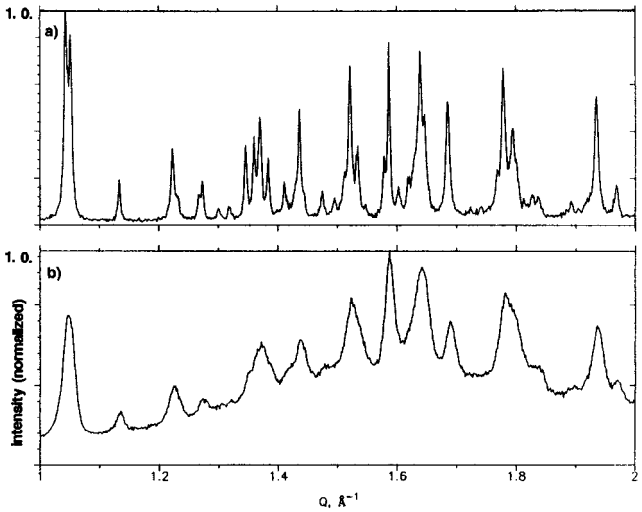

Figure 5—A partial comparison of a (a) synchrotron pattern of form II collected using a wavelength of 1.00006 Å with a (b) conventional X-ray diffraction pattern using Cu Kα radiation in a region where there are many overlapping peaks. The patterns are plotted as a function of $Q = 2\pi/d = 4\pi \sin \theta/\lambda$ to removal the effects of using different wavelengths.

routine in GSAS. The fit is shown in Figure 4b. The final monoclinic unit cell is:

	a	*b*	*c*	*β*	*V*
value:	4.992	54.77	9.372	99.15	2529.9
σ:	0.002	0.02	0.003	0.04	1.5

Conclusions

Conventionally, there are two ways to differentiate polymorphs: single-crystal or powder X-ray diffraction. The former requires fairly large crystals which, in many cases, are very difficult to obtain; the latter is often ambiguous if only visual comparisons of the patterns are made. Successful indexing of a powder pattern, however, strongly suggests that a pattern represents a single, pure phase and can be used as strong evidence for the existence of individual polymorph. We have used a combination of electron and X-ray diffraction techniques to differentiate the polymorphs of roxifiban by demonstrating that they have different, but closely related, unit cell parameters. To our knowledge, this represents the first time this combination of techniques has been used to differentiate two polymorphs of a pharmaceutical drug substance.

The success of this study was critically dependent on the use of a synchrotron diffraction pattern to obtain reliable indexing and accurate lattice parameters. Figure 5 compares the diffraction patterns of form II using (a) S-XRPD and (b) commercial X-ray diffractometer (C-XRD). The much higher resolution of the S-XRPD pattern is clear: the separation of the nearly overlapped peaks was critical to the indexing and to the refinement of the unit cell parameters of the two polymorphs.

Given that the patterns match so closely in the low-angle region, it is not surprising that the unit cell parameters of forms I and II are quite similar. The two extra peaks found in the low-angle region of form II are a consequence of the fact that the *b*-axis, and hence the unit cell volume, is approximately double that of form I. A schematic representation of the two unit cells is shown in Figure 6. It is reasonable to suggest that the long axis of the molecules must be aligned with the *b* axis in both cases. In fact, given that the form I cell is triclinic with space group *P*1 (and hence the origin of the cell is arbitrary), it might be possible to determine its structure by placing a pair of "optimized"

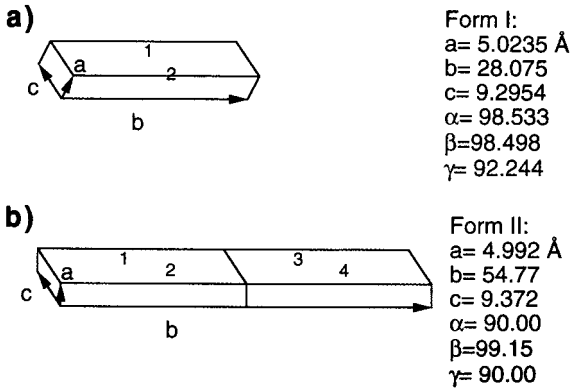

Figure 6—Schematic representation of unit cells of (a) form I with two molecules per cell and (b) form II with four molecules per cell. The molecules presumably align themselves approximately parallel with the very long *b*-axes.

molecules of roxifiban in the unit cell and adjusting their orientations. The task would be complicated by the number of flexible torsion angles in the molecule and by the fact that it is actually a salt, but it may be possible.

Because most crystallites which fail to grow to sizes that would make them amenable to routine single-crystal diffraction techniques have either platelike or needlelike habits, this combined use of TEM and synchrotron powder diffraction techniques should be applicable to similar structural problems in the pharmaceutical industry. Although access to synchrotron powder diffraction beamlines is presently somewhat limited, the use of synchrotron data for the differentiation of these two polymorphs was absolutely critical to the success of this study. In the future, access to such beamlines will be more readily available as more synchrotrons come on line and as better detector systems for powder diffraction are developed.[8]

References and Notes

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7. Program to visually display a measured powder diffraction pattern and to overlay it with the calculated positions of peaks derived from the unit cell dimensions. As the cell dimensions are altered, the calculated positions are adjusted in real time. Further information can be obtained from one of the authors (B.H.T.).
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Acknowledgments

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