Polymorphism

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Studying Microstructure in Molecular Crystals With Nanoindentation: Intergrowth Polymorphism in Felodipine**

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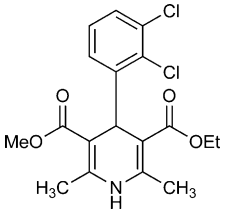
Abstract: *Intergrowth polymorphism refers to the existence of distinct structural domains within a single crystal of a compound. The phenomenon is exhibited by form II of the active pharmaceutical ingredient felodipine, and the associated microstructure is a significant feature of the compound's structural identity. Employing the technique of nanoindentation on form II reveals a bimodal mechanical response on specific single-crystal faces, demonstrating distinct properties for two polymorphic forms within the same crystal.*

The physical, chemical, and mechanical properties of materials depend on their crystal structures as well as the microstructures assumed during different stages of processing and handling. Understanding such structure–property correlations—and in turn applying the principles to design new materials—is a cornerstone of materials science and engineering. For molecular organic compounds, especially pharmaceuticals that need such an approach to enhance their applicability,[1] detailed understanding of structure–property correlations can be hindered by difficulties applying techniques that are routinely employed for inorganic compounds (e.g. electron microscopy), and diffraction and/or spectroscopy are largely relied upon. The general inability of X-ray diffraction (XRD) to resolve microstructural heterogeneities is a limitation.

One example of microstructural heterogeneity that can exist in molecular crystals is related to polymorphism, referring to different crystal structures of the same chemical compound. The notion that polymorphs are distinct structural entities, each with its own distinctive XRD pattern, is challenged by examples where dynamics,[2] disorder,[3] and chemical effects[4] come into play, such that a crystal may in some cases be more accurately described as a combination of different structural domains. One such case is aspirin,[5] for which it has been shown that single crystals can contain domains with a structure corresponding to the long-known polymorph I, and domains with a structure corresponding to the closely related, but distinct, polymorph II. We refer to this situation as "intergrowth polymorphism", to reflect the fact that a given single crystal is neither one polymorph nor the other, but an intergrown mixture of the two. In that case, the associated microstructure is a significant feature of the compound's structural identity.

Intergrowth polymorphism can be indicated by features in a single-crystal XRD pattern,[6] but detailed characterization of the microstructure generally requires an independent technique. One possibility is nanoindentation, which can establish the mechanical properties of single crystals with high precision, and which has shown its utility in crystal engineering.[7] In the context of intergrowth polymorphism, an advantage of nanoindentation over XRD is its superior spatial resolution: a nanoindenter's sharp tip usually has a radius close to 100 nm, while a typical (microfocus) X-ray beam has a diameter around 100 μm. Thus, nanoindentation can probe a crystal face with sufficient spatial resolution to seek microstructural features not accessible by XRD. For example, when applied to aspirin, nanoindentation could identify regions with a response indicative of form I in crystals that appeared by XRD to be solely form II.[8] Study of intergrown aspirin crystals (as per XRD) yielded a variety of intermediate responses, consistent with a complex microstructure.

Herein, we report that crystals of the active pharmaceutical ingredient (API) felodipine (form II; Scheme 1) exhibit intergrowth polymorphism and demonstrate that the compound's microstructure leads to a clear bimodal nanoindentation response. Conceptually, this is a step forward compared to the earlier work on aspirin, because it shows conclusively that different domains of an intergrown crystal can exhibit signature properties, as well as different structures, indicative of truly distinct polymorphic forms. This has implications for identification (not least in a legal/regulatory context) and structure–property correlations in molecular crystals.



Scheme 1. Felodipine.

Felodipine was first stated to be polymorphic in 1992,[9a] and two forms were characterized in 2001.[9b] Crys-

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tallization of form II is less reproducible than form I, but a method was described in 2009 by Lou et al., based on attempted co-crystallization with isonicotinamide.[9c] Further crystallization studies[9d] later identified two more felodipine polymorphs (forms III and IV), with form IV being obtained only once as a few single crystals. For form II, the published single-crystal structure is sub-optimal ($R1 = 0.100$, $wR2 = 0.243$),[9c] and it was subsequently suggested that form II crystals could be prone to layer stacking disorder.[10] We have now examined numerous crystals of felodipine form II and found consistently that single-crystal X-ray diffraction images contain split reflections. Standard indexing procedures typically identify two different unit cells, depending on the data that are selected. One of these (cell **A**; Table 1) corresponds

Table 1: Crystallographic unit cells for felodipine form II.

Centering	Lou et al.[9c] C-centered	Cell **A**[a] Primitive	Cell **B**[a] Primitive
a [Å]	32.392	18.705	18.705
b [Å]	18.717	18.705	18.705
c [Å]	23.711	23.711	25.621
α [°]	90	89.13	78.68
β [°]	91.0	89.13	67.72
γ [°]	90	60.04	60.04
V [Å^3]	14373.3	7186.3	7186.3
Z[b]	32	16	16

[a] Cell **A** is an equivalent primitive version of the *C*-centered cell. Cell **B** is a new form. Both **A** and **B** are presented as idealized versions obtained by exact transformation of the *C*-centered cell (see Supporting Information). Our experimentally obtained values are available in the Supporting Information. [b] Number of molecules in the unit cell.

to a primitive version of the published *C*-centered unit cell. The other (cell **B**) is a new form, identical to cell **A** in its *ab* plane but with a different *c* axis. The relationship between the unit cells is illustrated in Figure 1 and further details of the XRD pattern are provided in the Supporting Information.

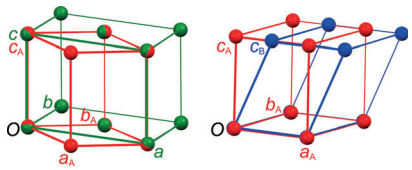

Figure 1. Relationship between the unit cells. Green = *C*-centered cell published by Lou et al.[9c] Red = cell **A**. Blue = cell **B**. Cell **B** is related to cell **A** by shearing so that $c_B = c_A + {}^1/_2 a_A$. An equivalent cell **B** in an alternative orientation (not shown) can be made so that $c_B = c_A + {}^1/_2 b_A$.

Using cell **A**, the crystal structure can be solved from our XRD data to yield a result equivalent to that published by Lou et al.[9c] Using cell **B** provides a closely comparable, but distinct, structure. The structures contain identical two-dimensional layers (i.e. they are polytypes[11]), but the stacking pattern is such that every fourth layer does not overlay. The non-overlapping layers are offset with respect to each other by a translation of ${}^1/_2 a$ (or ${}^1/_2 b$) in the primitive unit cell. For most crystals that we examined, the structure with cell **A** could be refined to about the same standard as that reported by Lou et al. (our data: anisotropic atoms, $R1 = 0.085$, $wR2 = 0.257$), while the alternative structure with cell **B** provided a less satisfactory result (isotropic atoms, $R1 = 0.129$, $wR2 = 0.363$).[*] The relatively poor agreement factors stem principally from systematic errors introduced by ignoring the contribution of the other polymorphic form in the XRD pattern.[5a]

The core element of the structure is a two-dimensional layer parallel to the crystallographic (001) planes (Figure 2), comprising pairs of felodipine molecules with a back-to-back arrangement of their 1,4-dihydropyridine rings. The molecules are linked by N–H···O hydrogen bonds into ribbons that propagate along the primitive unit-cell axes, and molecules within each hydrogen-bonded ribbon are related by translation with length equal to half that axis (Figure 2). In crystallographic terms, this is referred to as a pseudo-translation, because it is a local symmetry operator rather than an operator that applies to the crystal structure as a whole. A schematic illustration of the structure is given in Figure 3. In the figure, layer (1) has its pseudo-translation along the *b* axis, layer (2) along the *a* axis, etc. Layer (3) can be placed in two alternative positions related by the ${}^1/_2 a$ pseudo-translation present in layer (2). The alternative positions form locally equivalent intermolecular contacts, but they have different translational relationships with respect to layer (1). Conceptually, this is comparable to the well-established *ABA* and *ABC* stacking sequences for the close packing of spheres. In principle, redefining the unit cell as shown in Figure 3 also changes the relative position of layer (4), but since layer (4) has pseudo-translation parallel to *a*, the change is not apparent, and the structures differ only at every fourth layer. A similar description can be made by considering layer (2) displaced by ${}^1/_2 b$, to produce an equivalent structure with cell **B** in an alternative orientation. The structures described by cells **A** and **B** are distinct—they are different polymorphs (polytypes).

We have carried out nanoindentation on single crystals of the three accessible felodipine polymorphs (form IV being irreproducible to date). Forms I and III do not exhibit any intergrown properties but they are included for comparison. The measured values of elastic modulus (E) and hardness (H) for the indented crystal faces are listed in Table 2.[**] The crucial result is that the {100} face of form II exhibits a clear bimodal response. Figure 4 shows a plot of E versus H for

[*] The cell **B** structure appears to be twinned on account of the alternative shearing directions along either *a* or *b*. The quoted figures-of-merit include modelling of this twinning. CCDC 1012142–1012144, contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[**] The faces of form II used for nanoindentation are labelled with reference to the *C*-centered cell (Table 1), since this provides an approximately orthogonal basis. Indentation on {001} applies the indenter normal to the plane of the (001) layers, and indentation on {100} applies the indenter parallel to the plane of the (001) layers (see Figure 2).

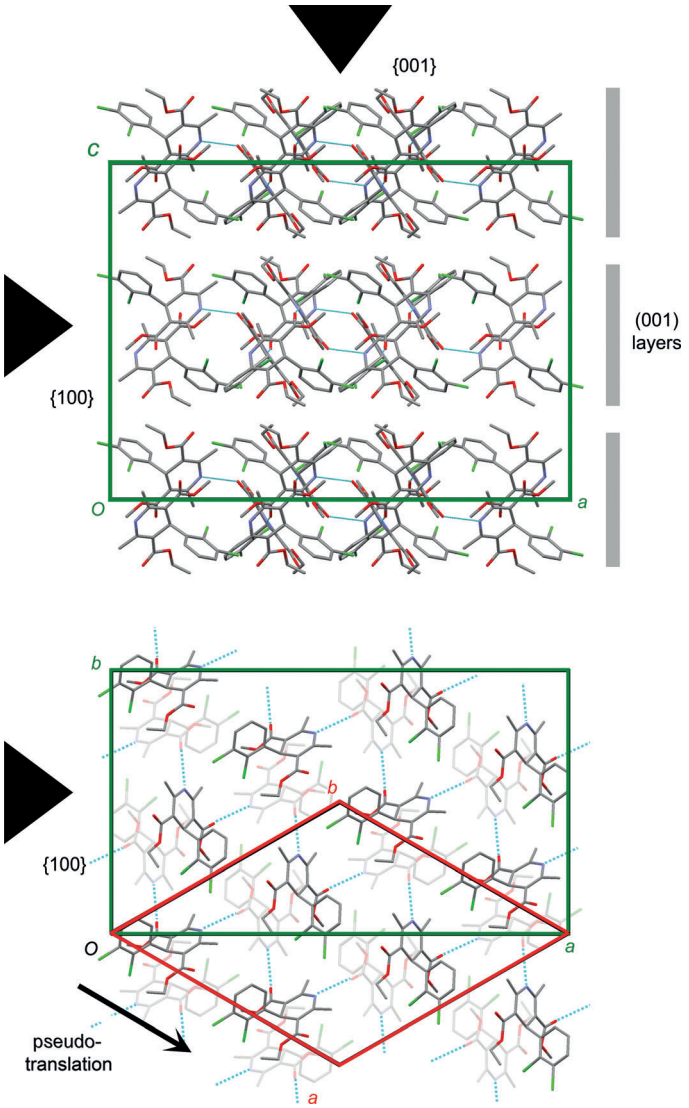

Figure 2. Top: view of the (001) molecular layers, showing the *C*-centered cell. Bottom: projection onto one (001) layer showing the primitive (red) and *C*-centered (green) unit cells. Pseudo-translation along the primitive *a* axis is indicated. Blue dashed lines indicate N–H···O hydrogen bonds. H atoms are not shown. The applied nanoindentation directions are indicated by the large black arrowheads.

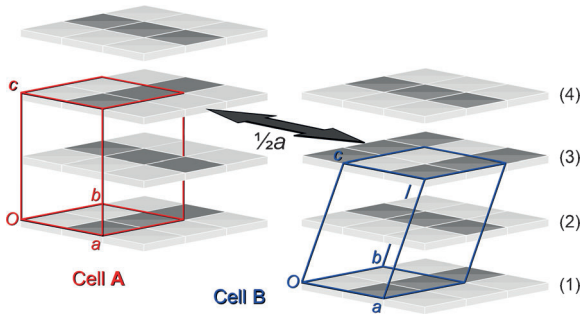

Figure 3. Schematic representation of the felodipine form II structure. Regions with identical shading represent molecules related by pseudo-translation. The indicated $^{1}/_{2}a$ displacement of layer (3) transforms cell **A** (red) to cell **B** (blue). In the stack of four layers shown, the two structures differ only in the position of layer (3). A movie illustrating the relationship between the two structures is included in the Supporting Information.

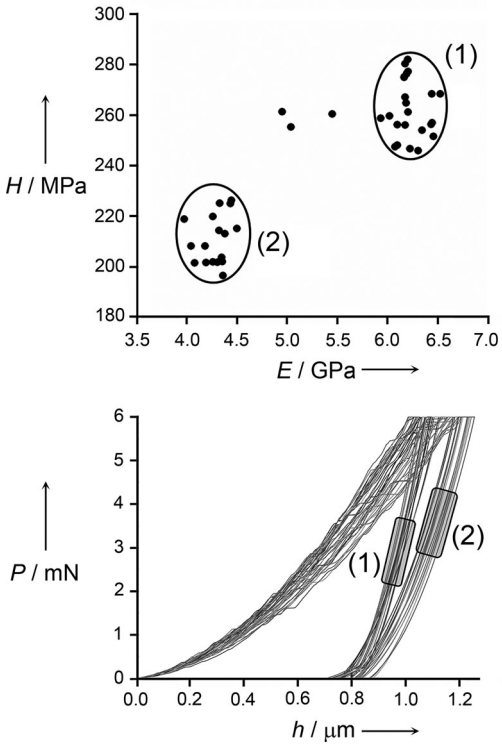

Figure 4. Top: plot of hardness (*H*) versus elastic modulus (*E*) for nanoindentation on the {100} face of felodipine form II. 42 indents are represented, obtained from three different single crystals. Bottom: corresponding load–displacement (*P*–*h*) curves. Discrete displacement bursts ("pop-ins") are visible as horizontal steps in the loading part of each curve.

42 indents on the {100} face of three different form II crystals, which lie in two distinct clusters, labelled $\{100\}_{(1)}$ and $\{100\}_{(2)}$ (plus three outliers). The corresponding load–displacement (*P*–*h*) curves can also be seen to form two different sets. All of the other indented faces on forms I–III, including the {001} face of form II, show a standard unimodal response (Table 2 and Supporting Information). The bimodal response is observed for multiple indents on the {100} face of an individual single crystal, so it is a property of each form II single crystal rather than any variation between different crystals. The two values for *E* and *H* listed in Table 2 are average values for the data clusters indicated in Figure 4, with the three outliers omitted. The variances of the values are comparable to the variances obtained for the unimodal indents on all of the other crystal forms (Table 2), which shows that they represent distinct, well-defined responses, as expected from two different polymorphs. The response labelled $\{100\}_{(1)}$ is harder and stiffer than $\{100\}_{(2)}$. The three outlier measurements could correspond to indents that simultaneously sample different polymorphic domains (i.e. close to a domain boundary), or more disordered regions.

Additional information in the nanoindentation loading curves is obtained from the "pop-ins", which correspond to

Table 2: Elastic modulus (*E*) and hardness (*H*) for felodipine single crystals.

Crystal	*E* [GPa]			*H* [MPa]		
	Value	Std. dev.	Coeff. of variation [%]	Value	Std. dev.	Coeff. of variation [%]
Form I {100}	12.81	0.19	1.5	553	16	2.9
Form II {001}	6.24	0.18	2.9	258	7	2.7
Form II $\{100\}_{(1)}$	6.19	0.25	4.0	260	10	3.8
Form II $\{100\}_{(2)}$	4.29	0.13	3.0	208	8	3.8
Form III {10-1}	10.47	0.19	1.8	451	13	2.9

discrete plastic displacement bursts. Statistical analysis of the pop-in magnitude (see Supporting Information) shows differences for the two {100} responses. For $\{100\}_{(1)}$, the pop-ins occur with displacement magnitudes of approximately 16, 32, and 48 nm, while the observed displacement magnitudes for $\{100\}_{(2)}$ are approximately 8, 16, and 32 nm, with some larger displacements (up to 80 nm) also seen. The displacements for $\{100\}_{(1)}$ correspond to multiples of a whole $\langle 100\rangle$ primitive lattice vector (which lies at 30° to the indenter direction: $18.717 \times \cos(30°) = 16.209$ Å), while those for $\{100\}_{(2)}$ correspond to half of a $\langle 100\rangle$ primitive lattice vector. The half-integral displacements for $\{100\}_{(2)}$ must transform the softer form into the harder form. The values of *E* and *H* derived for $\{100\}_{(1)}$ are essentially identical to the {001} response, but the differences in the pop-ins confirm that the responses are distinct and that they correspond to the indicated directions. The polymorph corresponding to $\{100\}_{(1)}$ therefore exhibits an essentially isotropic response in the two tested directions, while the polymorph corresponding to $\{100\}_{(2)}$ shows a more anisotropic response. The unimodal response along {001} indicates that the different domains show comparable behavior on indentation perpendicular to the consistent (001) layers.

In the current context, it is not of primary importance to link the $\{100\}_{(1)}$ and $\{100\}_{(2)}$ responses explicitly to the cell **A** or cell **B** structures. The close similarity between the two structures makes this a difficult task, and we have not yet resolved the issue. Possibly it could be best achieved through some high-level computational method.[13] It is sufficient to note that nanoindentation is established as a fingerprinting technique for polymorphs,[7b,8,12] so the bimodal response clearly confirms the intergrown microstructure.

These results for felodipine form II establish conclusively that intergrowth polymorphism is a genuine phenomenon in molecular crystals—the apparently unusual earlier case of aspirin is not an isolated curiosity. The observation of domains with distinct structures and distinct properties within apparently single crystals leaves no doubt that the crystal's microstructure is a significant feature of its structural identity in these intergrown cases. It is shown that nanoindentation provides a powerful technique to probe such features.

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