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The relation between twinning and disorder in the γ form of pyrazinamide

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1. INTRODUCTION

Pyrazinamide (PZA), an active pharmaceutical ingredient (API) is a first-line drug for the treatment of tuberculosis.[1] Pyrazinamide possesses at least four known polymorphs, named α ($P2_1/n$), β ($P2_1/c$), γ (Pc) and δ ($P\bar{1}$).[2-5]Among these polymorphs, the γ form exhibits a distinctly different packing from the other three polymorphs as one intermolecular hydrogen bond (N-H···N) links the amide group with the pyrazine ring forming linear chains of single H-bonded molecules.[6] The γ form is the most stable polymorph above 119 °C.[3,7] Single crystals of γ can be obtained by sublimation or by direct transformation of an α single crystal (although the latter method does not appear to be trivial).[3,6]

In the literature, disorder in crystals of the γ form has been reported, [4,6] taking the form of two molecular orientations in which the pyrazine ring and the amide moiety of the disordered molecules have been exchanged. It has not been clear so far whether the disorder may be static or dynamic. A considerable number of γ form crystals obtained by sublimation exhibits twinning, behavior that has been reported for the α form,[8] but had not been noticed so far for the γ form. The combination of disorder and the multitude of observed twinned crystals naturally brought the question to the fore whether twinning and disorder are related in the γ form, which is the subject of the present study.

2. MATERIALS AND METHODS

2.1 Materials

Pyrazinamide (PZA, $C_5H_5N_3O$, 123.11 g mol^{-1}, purity ⩾97.5% (GC)) was purchased from Sigma-Aldrich (France) and used without further purification. The polymorph of commercial PZA is α and its polymorphic purity was confirmed by X-ray diffraction. 1,3-Dimethylurea (DMU, $C_3H_8N_2O$, 88.11 g mol^{-1}, purity ⩾98 %) was purchased from Merck KGaA, and used without further purification. Tetramethylurea (TMU, $C_5H_{12}N_2O$, 116.16 g mol^{-1}, purity ⩾99% (GC)) was purchased from Alfa Aesar and used without further purification.

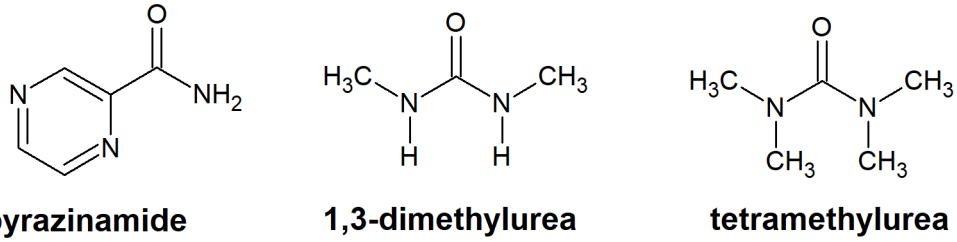

Figure 1. Molecular structures of pyrazinamide, 1,3-dimethylurea, and tetramethylurea

2.2 Preparation of the γ form

Crystals of the γ form were obtained by sublimation. The setup for their preparation is shown in Figure 2. 30 mg of commercial PZA was introduced into the larger vial and for a number of

samples an additive (DMU or TMU) was introduced into a much smaller vial. The larger vial was sealed before it was put into the oven. Samples without additives were kept at 124 °C for 96 h, which was reduced to 24 h with TMU. In the presence of DMU, samples were kept at 120 °C for 24 h. The obtained crystals were analyzed under the microscope and used for further characterization.

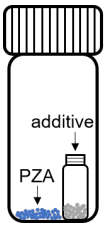

Figure 2. Schematic of the sample environment for the preparation of γ form crystals in the presence of additives

2.3 Single Crystal X-ray diffraction (SCXRD)

Single crystals used for SCXRD were obtained by sublimation in the presence of 1,3-dimethylurea. These crystals are overall of better quality than crystals obtained in the absence of additives or in the presence of TMU. X-ray Diffraction measurements were carried out at different temperatures. A Bruker D8 Venture 4-circle diffractometer with a Photon II detector (Germany) was used with monochromatic Mo$K\alpha$ radiation (λ = 0.71073 Å) equipped with an Oxford cooling device (UK). Heating was carried out by placing the diffracting crystal inside an oven. The heavy atoms were located using direct methods with the program Apex 3 of Bruker.[9] The H atoms were localized by successive difference Fourier maps with SHELXL implemented in the WinGX software package.[10,11]

3. RESULTS AND DISCUSSION

3.1 Crystal images and X-ray diffraction precession plots

Crystals of the γ form obtained by sublimation experiments in the absence and presence of TMU were analyzed under a polarized light microscope and for the two experimental conditions both single and twinned crystals were found. Their habits are presented in Figure 3. As the twinned domains interact differently with polarized light, single and twinned crystals can be distinguished and separated by polarized optical microscopy.

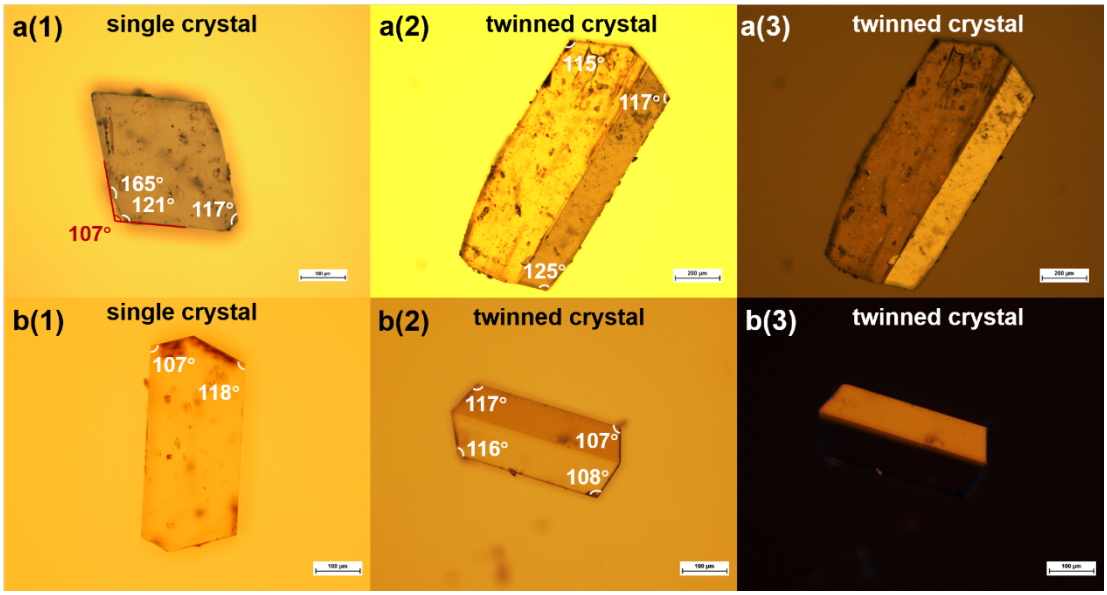

Figure 3. Crystal habit of PZA γ form crystals **(a)** in the absence of TMU and **(b)** in the presence of TMU. **(1)** single crystals, **(2)** twinned crystals under normal light and **(3)** under polarized light.

Precession plots of a single and a twinned crystal have been obtained and the [hk0] direction is presented in Figure 4. Directions [0kl] and [h0l] do not exhibit any clear indication of twinning; however, the [hk0] precession plot contains the tell-tale splitting of diffraction spots indicative of closely aligned twins.

3.2 Disorder

Disorder in the γ form manifests itself through two orientations of the pyrazinamide molecule on its single Z' = 1 site in the asymmetric unit (Figure 5).[4,6] The unit cell parameters of the γ form have been determined by single crystal X-ray diffraction as a function of temperature. The occupancy factor of the two orientations as a function of the temperature has been determined to be constant and the mean orientational occupancy was consistently found to be 0.79(1) over the entire temperature range from 110 to 450 K.

Cherukuvada et al. prepared single crystals of the γ form in solution at room temperature and carried out SCXRD at 100 K. They found a disorder site occupancy of 13%.[4] Wahlberg et al. prepared a single crystal of the γ form by transforming a single crystal of the α polymorph at around 420 K and crystallographic data were collected at 122 K. The disorder site occupancy was found to be about 20%.[6] In the current experiments, crystals have been obtained by sublimation at around 390 K. The disorder site occupancy was found to be 21%. Hence, the site occupancy may be related to the crystallization method in combination with the temperature during crystallization; however, an overall tendency for disorder exists in the γ form. As the disorder site occupancy is independent of the temperature, the disorder is static.

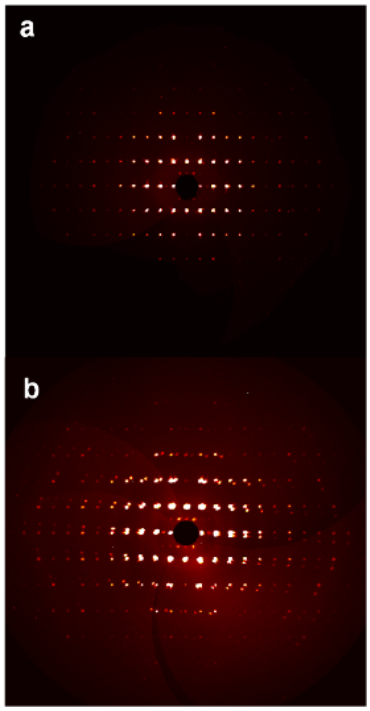

Figure 4. X-ray diffraction precession plots of the [hk0] direction measured for (a) single and (b) twinned γ form crystals

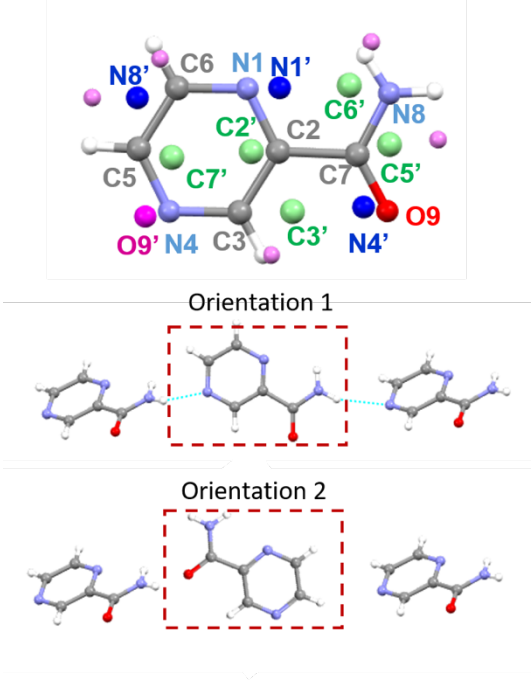

Figure 5. Disorder in form γ: two existing molecular orientations (around 79% of occurrence for orientation 1 and around 21% for orientation 2)

3.3 The twinning interface

The calculated morphology of PZA γ form crystals and the orientation of the pyrazinamide molecules in relation to certain key crystal faces are shown in Figure 6. Typical experimental twinning habits are shown in Figure 7b when grown in the absence of additives and c when grown in the presence of TMU (see Figure 3 for more examples). Through the angles between the crystal faces and using the calculated crystal morphology, it has been possible to determine the twinning configuration (Figure 7a). Compared to the calculated twinning morphology, the experimental one obtained in the absence of additives (Figure 7b) exhibits some differences. Firstly, the (-102) or (10-2) face, marked by a green star in Figure 7, becomes larger and almost completely displaces the adjacent face (002)/(00-2), which makes an angle of 107° with the twinning interface. Secondly, a distinct difference with the theoretical twin is that the angle between the two faces (00-2) and (10-2) (or faces (002) and (-102)) marked by the red line in Figure 7 appears to become almost straight. The morphology of twinned crystals in the presence of tetramethylurea, on the other hand, does not show such a

change and the angles remain the same as those in the ideal morphology (Figure 7c), even if on each side of the crystal one of the "adjacent" faces completely disappears.

Based on the angles in the twinned crystals, and the possible arrangement of pyrazinamide molecules at the twinning interface (e.g., head-tail, head-head or tail-tail configurations) three twinning configurations can be proposed as shown in Figure 8 (Face (10-2) is marked in red). As remarked above, the growth rate of the faces in twinned crystals is different from that observed for single crystals; the two domains grow symmetrically with the interface as a mirror plane. This observation excludes twinning scenario 1 (Figure 8).

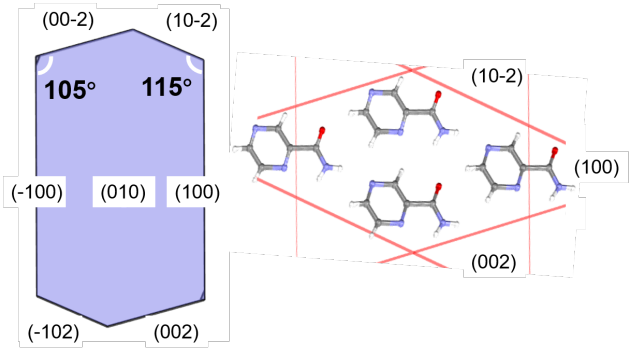

Figure 6. Calculated morphology of PZA γ form crystals and packing of the γ form in relation to the key crystal faces.

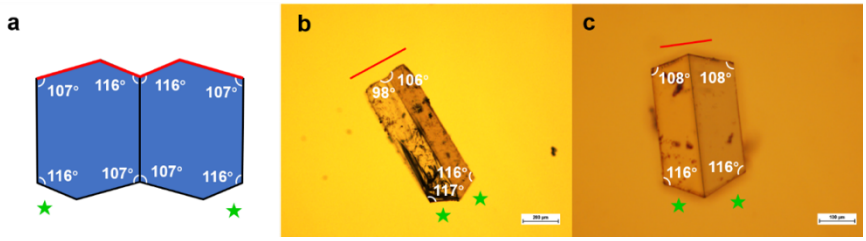

Figure 7. Typical twinning morphology: (a) ideal, (b) in the absence of additives, (c) in the presence of tetramethylurea

For twinning scenario 2, the interface would be (-100). The faces bordering (-100) are (00-2) and (-102). For twinning scenario 3, the interface would be (100) and on the sides would be faces (002) and (10-2). Amide oxygen, which is the highest electron density region, is exposed on the faces (100) and (10-2). The amide hydrogens, which are poor in electrons, are exposed on the faces (100) and (002). Due to these electron density differences, pyrazinamide single crystals are polar, whereas in scenario 3, the growth along the twinning interface reduces the size of the (10-2) face leaving the oxygen atoms less exposed and the same occurs for the amine groups on the (002) faces at the other side of the twinning interface. As a result, the overall dipole of the twinned crystal is reduced, and this may explain the disappearance of these two faces. In scenario 2, an opposite effect occurs in which faces (10-2) and (002) will be part of the twinned crystal, implying that the overall dipole of the twinned crystal may increase. The twinning interface is therefore most likely the (100) face with the amide groups of both domains facing each other. The proposed twinning configuration can be observed in Figure 8(3). Although in this presentation the oxygen atoms appear juxtaposed, on the twinning plane, these groups are angled and can shift along the twinning plane to form hydrogen bonds with the amide hydrogens, making scenario 3 even more likely.

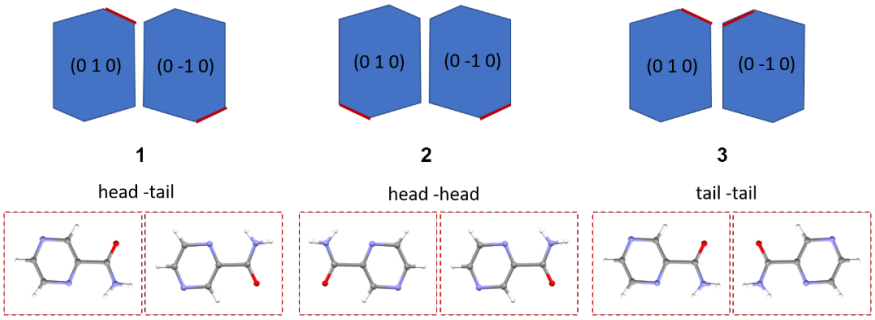

Figure 7. The three possible twinning configurations based on the observed angles (surface (10-2) is marked in red) and the packing arrangement of two pyrazinamide molecules at the twinning interface.

3.4 Relationship between disorder and twinning

For crystals without disorder, a pyrazinamide molecule possesses twelve short contacts (Figure 9a, the data is obtained from X-ray diffraction measurements on γ single crystals performed at 294 K), which are effectively six short contacts per molecule. The strongest interaction is the N-H···N hydrogen bond with an H···N distance of 2.305 Å (3 and 8 in Figure 9b). For the molecule with orientation 2, the short contacts are shown in Figure 9c. Some of these contacts are even shorter than those in the ordered crystal: O···H-N with 2.051 Å (2' in Figure 9c), N-H···O with 2.147 Å (1' in Figure 9c) and C-H···O with 2.283 Å (9' in Figure 9c). Thus, disorder gives rise to relatively strong interactions between the amide groups, which is the main motif in the other three polymorphs of pyrazinamide, while partially compensating the dipole moment over the crystal. The physical reason behind this is that the disorder diminishes the polar aspect of the crystal, because the dipole of the disordered molecules has turned. Thus, disorder and twinning appear to have the same cause in the γ form of

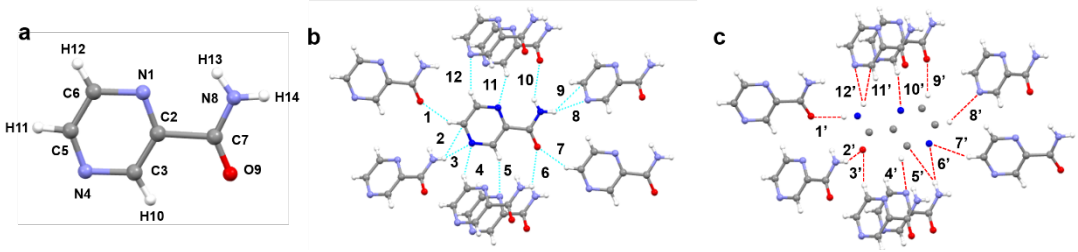

Figure 8. Short contacts between the pyrazinamide molecules in the γ form: **(a)** molecular structure of pyrazinamide, **(b)** short contacts in the γ form with molecule of orientation 1, **(c)** short contacts in the γ form with molecule of orientation 2

pyrazinamide.

As has been concluded just above, twinning is in part a means for the crystal to compensate its overall dipole moment. In addition, the interface is reinforced by a network of strong hydrogen bonds between the amide groups. It would appear logical to see the disorder in the same light to decrease the overall dipole of the γ crystal, while the disordered molecules are kept in place by strong interactions.

As is shown in Figure 3, the presence of urea derivatives does not eliminate twinning but optimizes it. Most of the crystals obtained in the presence of TMU have a clear interface and only contain two domains. Hence, TMU cannot prevent twinning and it would be logical to assume that twinning is an inherent part of the molecular structure[12] as mentioned at the beginning of this section.

In the energy calculations by Wahlberg et al.[6], the disordered form was modeled as a separate crystal structure, which would basically be similar to a form of twinning. Nonetheless, the calculations by these authors are the best estimate for the energy of the disordered system. Three different models were constructed by the authors: model A consists of molecules in the ideal ordered orientation 1 (in Figure 5) with an occupation of 0.8 in the real crystal, model B consists of 100% of the molecules in the disordered orientation 2 (in Figure 5) and model C is the combination of A and B reflecting the observed disorder. Their result showed that the energy of model A was the lowest one, the energy of model B was 12 kJ mol^{-1} higher than model A and the energy of model C was 3 kJ mol^{-1} higher than model A. Adding entropy to this value (~1.65 kJ mol^{-1}), would bring the Gibbs energy difference between model C and A down to 3 - 1.65 = 1.35 kJ mol^{-1}. Considering that there may be a reasonable margin in the actual lattice energies calculated for the "pure" form and the disordered form, it can very well be that with the entropy, the disordered form might be the more stable one, possibly even just due to the entropic contribution.

4. CONCLUSION

In this paper, disorder and twinning of γ form of PZA have been investigated experimentally. The SCXRD results as a function of temperature shows that the disorder site occupancy is independent of temperature, and it suggests that the disorder is static. For twinned crystals, the morphology of each domain of the twinned crystals are different from that of single crystals. The twin interface is found to be (100) defining the orientation of the molecules on the twin boundary. The orientation of 'disordered' molecules and the orientation of the molecules on the twin boundary are the same, implying that both phenomena are closely related. Moreover, the disorder may be an inherent property of the crystal with a certain level of disorder leading to the lowest Gibbs energy overall for the crystal.

REFERENCES

[1] Zhang, Y. and Mitchison, D. (2003) The curious characteristics of pyrazinamide: a review, *The international journal of tuberculosis and lung disease* 7: 6-21,

[2] Rajalakshmi, G., Hathwar, V.R. and Kumaradhas, P. (2014) Intermolecular interactions, charge-density distribution and the electrostatic properties of pyrazinamide anti-TB drug molecule: an experimental and theoretical charge-density study, *Acta Crystallographica Section B* 70: 568-579,

[3] Castro, R.A., Maria, T.M., Évora, A.O., Feiteira, J.C., Silva, M.R., Beja, A.M., Canotilho, J. and Eusébio, M.E.S. (2009) A new insight into pyrazinamide polymorphic forms and their thermodynamic relationships, *Crystal Growth & Design* 10: 274-282,

[4] Cherukuvada, S., Thakuria, R. and Nangia, A. (2010) Pyrazinamide polymorphs: relative stability and vibrational spectroscopy, *Crystal Growth & Design* 10: 3931-3941,

[5] A.Nangia and A.Srinivasulu (2005) *CSD Communication(Private Communication)*

[6] Wahlberg, N., Ciochoń, P., PetriĉEk, V. and Madsen, A.Ø. (2013) Polymorph stability prediction: on the importance of accurate structures: a case study of pyrazinamide, *Crystal Growth & Design* 14: 381-388,

[7] Li, K., Gbabode, G., Barrio, M., Tamarit, J.-L., Vergé-Depré, M., Robert, B. and Rietveld, I.B. (2020) The phase relationship between the pyrazinamide polymorphs α and γ, *International Journal of Pharmaceutics* 119230,

[8] Takaki, Y., Sasada, Y. and Watanabé, T. (1960) The crystal structure of α-pyrazinamide, *Acta Crystallographica* 13: 693-702,

[9]

[10] Sheldrick, G.M. (2008) A short history of SHELX, *Acta Crystallographica Section A: Foundations of Crystallography* 64: 112-122,

[11] Farrugia, L. (1999) WinGX (Version 1.64. 05), *J. Appl. Crystallogr* 32: 837-838,

[12] Gracin, S. and Rasmuson, Å.C. (2004) Polymorphism and crystallization of p-aminobenzoic acid, *Crystal growth & design* 4: 1013-1023,