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Crystals, Cures and Conundrums

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X-ray powder diffraction (XRPD) is a powerful tool for the investigation of polycrystalline pharmaceutical solids, being widely applied to phase-identification (“fingerprinting”) of solid forms during experimental crystallisation searches during preclinical development screening [1], [2]. With the availability of modern diffractometers, yielding high quality data, plus free and commercial software tools, structure determination from powder diffraction data (SDPD) using global optimisation approaches, XRPD has also become widely used for structural analysis in the absence of suitable single crystal samples. As such, it is increasingly an important element of studies into novel molecular materials [3], polymorphs [4] and solvates [5]. Significantly, the structures of many pharmaceutical solids are complex comprising large, flexible molecules often with multiple fragments (co-crystals, salts, solvates), $Z > 1$ and possibly disorder. These features contribute to the overall complexity of the problem in terms of both the molecular (internal Degrees of Freedom, DoF) and crystallographic (external DoF) complexity. In order to maximise the chances of successfully locating the global minimum, particularly for such complex problems, a number of strategies can be adopted. Here we will focus, through the use of examples, on steps in the process that contribute to the successful, routine application of SDPD to challenging systems. This will include: data collection approaches; accurate input model generation; implementation of global optimisation calculations and the use of modal torsion angle constraints from the CSD [6]; robust Rietveld analysis and methods for final structure verification.

[1] G. Barr, W. Dong, C.J. Gilmore, *J. Appl. Cryst.* **2004**, *37*, 658-664. [2] A.J. Florence, et al., *J. Pharm. Sci.* **2003**, *92*(9), 1930-1938. [3] G.M. Espallargas, et al., *J. Am. Chem. Soc.* **2007**, *129*(50), 15606-15614. [4] P. Fernandes, et al., *Acta Crystallogr.* **2007**, *E63*, O202-O204. [5] P. Fernandes, et al., *J. Pharm. Sci.* **2007**, *96*(5), 1192-1202. [6] F.H. Allen, *Acta Crystallogr.* **2002**, *B58*, 380-388.

Keywords: powder diffraction data, pharmaceuticals, global optimization

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Solving Structures from Powder Data Using Maximum Entropy and Charge Flipping

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The solution of crystal structures from powder diffraction data has been revolutionized by the use of model building coupled with global optimization methods and simulated annealing [1]. The charge flipping technique for solving crystal structures [2] has also had a considerable impact in areas such as incommensurate structures, and situations where the space group is uncertain or unknown, and this method is also applicable to powder diffraction data. It is widely presumed that complete data to atomic resolution are needed for success with this technique, but with the incorporation of the maximum entropy

formalism [3], the method can be shown to work with data at *ca.* 2Å resolution. This combined approach works as follows:

1. Reflection intensities are extracted from the powder diffraction data using either the Pawley or the LeBail method.
2. The reflection data are passed to the *Superflip* computer program [4] which carries out charge flipping using the powder diffraction options. A total of 100 phase sets, starting from random phase choices, are generated and passed to the maximum entropy program *MICE* [5]. In general the solutions generated by Superflip with this data do not converge even after 10000 cycles and/or they have large figures of merit, and cannot be distinguished from each other.
3. Each phase set is subjected to constrained entropy maximization and an associated log-likelihood (LLG) figure of merit is calculated.
4. Electron density maps (in the form of centroid maps [6]) are computed for the top 24 solutions as ranked by LLG.
5. These maps are filtered by histogram matching [7], and the top 5-15 retained and examined.

The method has been used successfully on a number of powder XRD datasets collected from structures of varying molecular and crystallographic complexity of pharmaceutical importance [8]. In some favourable cases, maximum entropy alone (without the use of Superflip) can be successful. The solutions may well prove useful in defining approximate molecular envelopes that can be used as a starting point for model building methods.

The methodology also works for electron diffraction data including two-dimensional data sets.

[1] A.J. Florence, N. Shankland, K. Shankland, W.I.F. David, E. Pidcock, X. Xu, A. Johnston, A.R. Kennedy, P.J. Cox, J.S.O. Evans, G. Steele, S.D. Cosgrove, C.S. Frampton, *J. Appl. Cryst.* **2005**, *38*, 249-259. [2] G. Oszlányi, A. Süto, *Acta Cryst.* **2008**, *A64*, 123-134. [3] C.J. Gilmore, W. Dong, D.L. Dorset, *Acta Cryst.* **2008**, *A64*, 284-294. [4] L. Palatinus, G. Chapuis *J. Appl. Cryst.* **2007**, *40*, 786-790. [5] C.J. Gilmore, G. Bricogne, *Methods in Enzymology* **1997**, *277*, 65-78. [6] C.J. Gilmore, G. Bricogne, C. Bannister, *Acta Cryst.* **1990**, *A46*, 297-308. [7] C.J. Gilmore, W. Dong, D.L. Dorset, *Acta Cryst.* **2005**, *A64*, 284-294 [8] See: <http://www.powderdata.info/data.htm>

Keywords: powder diffraction, maximum entropy, charge flipping

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Macromolecular Powder Diffraction: From Structure to drug

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In recent years, modern powder diffraction techniques have been applied to several microcrystalline proteins. The use of high-resolution synchrotron data, together with new analysis procedures, has stimulated exciting progress which is showing that powders can offer unique opportunities for the structural characterization of proteins and are complementary to existing methods. The developments in experimental methods and instrumentation have been absolutely essential, and they will be discussed [1], [2], [3]. Perhaps the most enticing goal for any crystallographer is to solve new structures, and thus we will discuss the progress and prospects for powder diffraction data in this area. We will describe some of the structural refinements which have been carried out for powders using single or multiple pattern fitting and the Rietveld method [1]. The application of the powder diffraction method to phase identification in mixtures and crystal screening [4], [5] will also be illustrated.

[1] I. Margiolaki, et al. *J. Am. Chem. Soc.* **2007**, *129*, 11865–11871. [2] I. Margiolaki, et al. *Z. Kristallogr. Suppl.* **2007**, *26*, 1–13. [3] I. Margiolaki, J.P. Wright, *Acta Cryst. A* **2008**, *64*, 169–180. [4] I. Collings, et al. *Acta Cryst. D* **2010**, *66*, 539–548. [5] N. Papageorgiou, et al. *Z. Kristallogr.* **2010**, *225*, 576–580.

Keywords: powder diffraction, synchrotron radiation, protein crystallography

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Pseudo-polymorphic transition of pharmaceutical crystals revealed by SDPD method

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Many pharmaceutical crystals show pseudo-polymorphic transition via hydration / dehydration processes depending on their storage environment or mechanical treatment. Sometimes, even after dehydration, the XRD pattern does not change significantly showing “isomorphic desolvation” which attracted much interest. Also, as their physicochemical properties such as color, stability, and solubility largely differ depending on the crystal structures, the structural investigation of the transition is important especially to utilize the pharmaceutical polymorphic crystals as API. However, after the transition, single crystal integrity tends to degrade and powdery crystals are formed. In such case, *ab initio* Structure Determination from Powder X-ray Diffraction data (SDPD) is efficient technique. We have succeeded to reveal several solid-state structure rearrangement phenomena so far by using the technique [1-7].

Herein, some examples of the pseudo-polymorphic transitions relating “isomorphic desolvation” are presented, which have been investigated by SDPD technique.

Cephalexin (cephem antibiotic) has five hydrated forms and their reversible transformations are induced by the change of relative humidity. Three pseudo-polymorphs (anhydrate, monohydrate, and dihydrate) were successfully analyzed by SDPD technique. As the structure has three independent API molecules, the number of parameters was adjusted during the direct space calculations. Water molecules of hydration were located from the residual map, cavity volume, and geometrical considerations. The hydrate phases show water tunnel structure between L-shaped building blocks that were formed by three independent cephalexin molecules connected by hydrogen bonds. In the hydration process, the blocks slide each other to increase the tunnel volume from 0 to 280 Å³ (see figure), which is accompanied by elongation of the *a*-axis length by 17%. Such “sliding block” mechanism enables the large structural change with retention of crystallinity.

Erythromycin is used as a macrolide antibiotic drug in the dihydrate form. It released the water molecules at 355K or at dry condition to form anhydrate phase that has analyzed by SDPD technique. The structure (isomorphic desolvate) has void tunnel regions that were occupied by water molecules in the dihydrate phase. Thus, it should be transformed to more stable anhydrate structure.

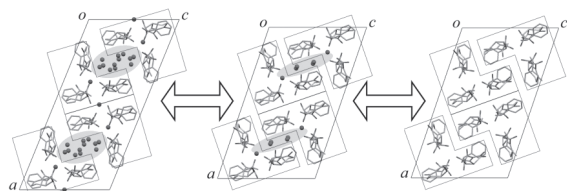


Fig. Cephalexin dihydrate, monohydrate, and anhydrate

[1] *Angew. Chem. Int. Ed.* **2006**, *45*, 6013, [2] *Int. J. Pharm.* **2009**, *369*, 12, [3] *Cryst. Growth Des.* **2009**, *9*, 1201, [4] *J. Phys. Chem. C.* **2010**, *114*, 580. [5] *Chem. Comm.* **2010**, *46*, 4264. [6] *Cryst. Growth Des.* **2010**, *10*, 2116, [7] *CrystEngComm.* **2011**, *13*, 3197.

Keywords: polymorphism, pharmaceutical, hydration

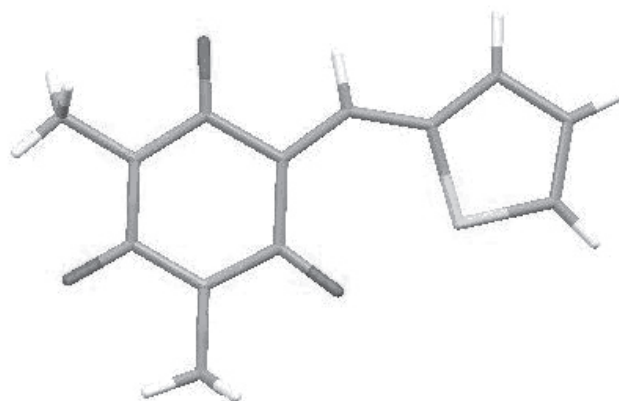
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Structure Determination of Barbiturate Derivative Using X-Ray Powder Diffraction

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The barbiturate derivative namely 1,3-dimethyl-5[(2-thienyl)methylene]-2,4,6-pyrimidinetrione (C₁₁H₁₀N₂O₃S) has numerous biological activities as anti-inflammatory, anticonvulsant and antibacterial. The molecular structure of this compound has been determined by the method of simulated annealing as implemented in Dash program from high resolution laboratory X-ray powder diffraction data collected at ambient conditions. It has been found that the compound crystallize in the monoclinic space group *P2₁/c* with lattice parameters *a*=7.2384Å, *b*=13.2319 Å, *c*=13.8221 Å, β =123.74° and unit cell volume=110.89 Å³. The crystal structure was refined using Rietveld refinement method on a data collected at 1.5 Å resolution yielded R-Bragg values of 7.91% and R_{wp} value of 6.4%. The molecules are stacked in parallel layers and are stabilized by hydrogen bonds.



Keywords: structure_determination, x-ray_diffraction, antibacterial

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Accurate mass, models and resolution for high-throughput structural analyses

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Efficiently characterizing biologically relevant conformations of macromolecules and their complexes is a critical challenge for