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Synchrotron X-ray Diffraction of the Structure of Amorphous Indomethacin Using PDF Analysis and EPSR Modeling

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PURPOSE

To enhance the solubility of orally administered pharmaceuticals, liquid or amorphous formulations are often preferred over crystalline drug products. However, little is known regarding the variation in bonding mechanisms between pharmaceutical molecules in their different disordered forms. High energy x-ray data combined with the x-ray pair distribution function (PDF) method is a powerful technique for the characterization of variations in both intra-molecular and inter-molecular packing arrangements. Advanced modeling techniques such as empirical potential structural refinement (EPSR) are revolutionary for visualizing the 3D arrangement of the molecules in disordered forms. Here, we expand upon our recent analysis of Indomethacin [1] by including new results using EPSR to further investigate the variation in hydrogen bonding between different amorphous forms.

METHODS

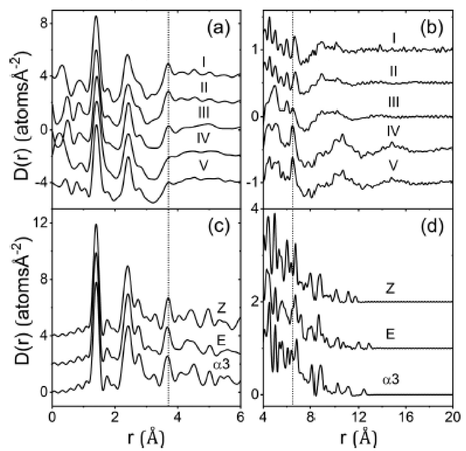
Powdered amorphous Indomethacin was loaded into 1.5 – 2 mm diameter capillaries and some were exposed to varying humidity levels during analysis. Scattering experiments were performed on a synchrotron beamline using an incident energy of 100 keV in transmission geometry. Diffraction data were collected on a Varex CT4343 area detector. The total x-ray scattering data were analyzed using Fit2D and PDFgetX2. EPSR simulations were performed on 64 molecules within a cubic box under periodic boundary conditions using atomic number densities of 0.090 atomsÅ^{-3} for sample II and 0.095 atomsÅ^{-3} for sample V. The starting configuration of the model was constructed from a random array of Z isomer molecules since this configuration is the most common, particularly at low density values. To introduce molecular flexibility, limited rotations of six molecular groups were enabled, including the rotation of the chlorobenzyl ring. Following initial Monte Carlo equilibration, the empirical potential term was refined to improve agreement with scattering data. Once the goodness-of-fit parameter was minimized between the model and the experimental S(Q), structural data were collected over ensembles of at least 10,000 configurations.

RESULTS

The Indomethacin molecule is comprised of a largely hydrophobic indole and chlorobenzyl groups and several hydrophilic groups: namely an amide, methoxyl, and a carboxylic acid that can act as donor or acceptor sites. γ-Indomethacin is the stable crystalline form which exists only as the Z isomer where hydrogen bonded dimers are connected through their carboxylic acid groups. α-Indomethacin is a denser metastable crystalline form comprised of three different isomers (Z, E and α3).

In our previous study of several amorphous samples, the x-ray PDFs were compared to models of the three different isomers (Figure 1). Samples I, II, and III showed a sharp peak at 3.7 Å whereas samples IV and V showed an absence of peaks at 3.7 Å, 4.4 Å, and 5.0 Å. These peaks can largely be attributed to the geometries of the hindered interactions of the chlorobenzyl ring with respect to the amide ring, suggesting a preferred geometry of the chlorobenzyl ring (more consistent with the known isomer orientations) in samples I, II, and III, and a nearly free rotation of the chlorobenzyl ring in samples IV and V. There is a competition between the hindered intra-molecular conformations associated with the preferred orientation of the chlorobenzyl ring (I, II, III) and the degree of inter-molecular hydrogen bonding (IV, V) in the amorphous forms [1]. Also, inter-molecular peaks are observed at ~10.5 and 15 Å for samples IV and V, indicating a greater amount of ordering of the molecules.

Figure 1. Five independently measured differential atomic pair-distribution functions D(r) for dry amorphous Indomethacin (a) and (b) compared to those calculated for the Z, E, and α3 isomers (c) and (d), offset for clarity.



Both samples II and V had been created by melt quenching the γ-form of Indomethacin in liquid nitrogen, yet their PDF curves were different. To better understand the differences between samples II and V, they were further studied by EPSR (Figure 2). In sample II, the first sharp diffraction peak (FSDP) was found to be of lower intensity than that of sample V. In sample II, the chlorobenzyl ring exhibits distinct isomer orientations resulting in a reduction of available hydrogen bonding options to other molecules. Conversely, sample V has no preferred torsion angles of the chlorobenzyl ring, enabling a greater diversity of hydrogen bonding interactions. The 20% increase in the intensity of the FSDP for sample V is consistent with this interpretation.

The EPSR partial PDFs provide a direct indication of the degree of hydrogen bonding in amorphous Indomethacin. The model for the less ordered sample (II) has a first oxygen-oxygen (O-O) peak at 2.43 Å and a broader second peak at 3.4 Å whereas the more ordered sample (V) has an intense peak at ~2.4 Å and a sharper second peak at 3.4 Å. This first peak represents the strong O-H-O hydrogen bonding between adjacent molecules. The second peak is due to weaker (longer) hydrogen O-H-O bonds (Figure 3). The running $n_{O1\text{-}O2}(r)$ coordination number for sample V begins sooner and is higher than sample II up to about 4 Å, indicating the atoms are closer and more strongly bonded in V (Figure 4). Lastly, the chain size (the number of molecules connected by hydrogen bonding) is presented in Figure 5. Sample II has more isolated (0) and one neighbor (1) molecules whereas sample V has larger chain sizes (2 and 3). These results support the conclusion that sample V is more ordered than sample II.

Figure 2. The x-ray structure factors of two amorphous Indomethacin samples (II and V), together with the EPSR model fits.

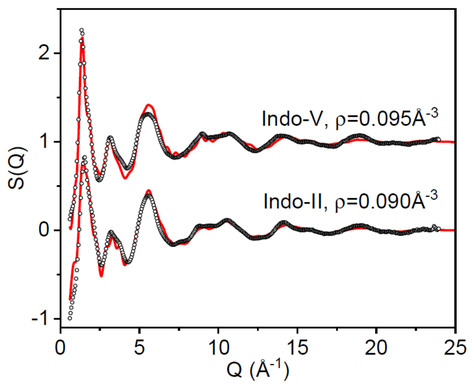


Figure 3. The inter-molecular O1-O2 partial structure factors from the EPSR models.

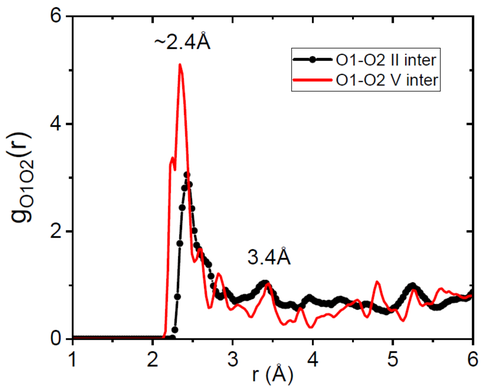


Figure 4. The corresponding running coordination number for amorphous Indomethacin samples II and V.

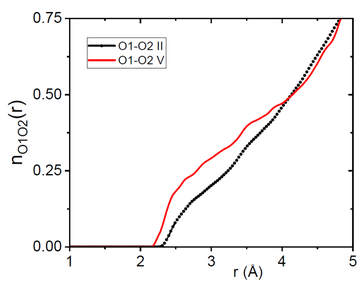
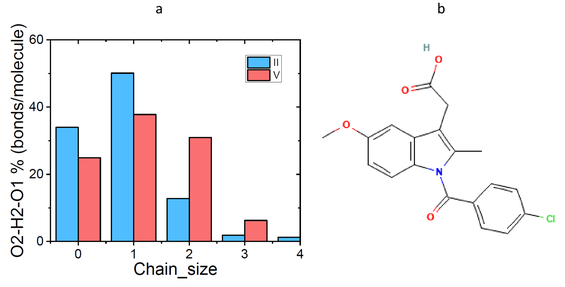


Figure 5. a) The number of hydrogen bonds per molecule as a function of chain size for amorphous Indomethacin samples II and V. b) the 2D structure of Indomethacin



CONCLUSIONS

In our previous work, the molecules in sample II were determined to have a preferred geometry of the chlorobenzyl ring more consistent with the known isomer orientations. The molecules in sample V were found to have a nearly free rotation of the chlorobenzyl ring. EPSR modeling was used to further investigate the variation in hydrogen bonding between these two amorphous Indomethacin samples. EPSR showed that most molecules are bonded to one neighboring molecule via a single hydrogen bond. The number of isolated molecules is higher in sample II. The intra-molecular structure is more ordered but there is no inter-molecular ordering with respect to hydrogen bonding. In sample V, there are more bifurcated hydrogen bonds leading to a diversity of chain structures in the amorphous forms. The intra-molecular structure is less ordered but there is more inter-molecular ordering with respect to hydrogen bonding. The freedom of the intra-molecular structure allows the molecule to move around and adopt more orientations that favor inter-molecular hydrogen bonding.

REFERENCE

[1] Benmore, C., et al., *A High Energy X-ray Diffraction Study of Amorphous Indomethacin.* Journal of Pharmaceutical Sciences, 2022. **111**(3), 818-824.

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