Atomic pair distribution function: a revolution in the characterization of nanostructured pharmaceuticals

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Given our druthers, we would never work with drugs in the amorphous (a-) or nanocrystalline (n-) solid forms. Like naughty, unruly children, they can’t be relied on and tend not to do as they are told: not traits that are desirable in the drug marketplace. But what if these were the only solid forms that a promising active pharmaceutical ingredient (API) could be brought to market? Or like the children, what if this form gave you exciting new possibilities, for example, for targeted drug delivery? Then it would be great to find foolproof ways of characterizing and controlling their undesirable excesses. Increasingly, we are pushed in this direction. Only a small percentage of drugs currently at market are amorphous. However, with 70–90% of the molecules in the drug development pipeline being classified as poorly soluble [1] in the biopharmaceutics classification scheme [2]. The increased solubility of the nanocrystalline [3] and amorphous [4,5] states is starting to look very attractive to formulation scientists.

To make substantial progress on these issues, it is critical to have tools that allow us to detect and characterize the structure of APIs in the a- and n-forms. This is a major challenge because the industry standard workhorse for structural characterization, conventional x-ray diffraction (XRD), loses its power when the crystallite size becomes nanometer in scale. The diffraction pattern gets broad and noisy, the so-called ‘amorphous halo’, and carries little information. However, recently, the development of the total scattering pair distribution function (TSPDF) method applied to small molecule systems has shown great promise for fingerprinting, quantification and even modeling of nanocrystalline and amorphous APIs.

The TSPDF method was developed for studying nanostructure in crystals [6] and was later applied to inorganic nanoparticles [7]. It grew out of a rich 70-year history of applying a similar approach to study the scattering from inorganic glasses and amorphous materials [8]. Coupled with modern developments in sources, instrumentation and computing, it is becoming a powerful method for studying structure at the nanoscale. It is based on a Fourier analysis of the x-ray, neutron and electron scattering data collected over the entire reciprocal space (from which it gets its ‘total scattering’ name) from an orientationally disordered sample [7]. More recently, it has been applied successfully to small molecule systems [9,10]. Earlier attempts to use similar Fourier methods on conventional XRD data [11] suffer from the lack of information in the conventional XRD pattern, and uncertainties in data processing and interpretation due to the narrow range of the data [12].

The total scattering pair distribution function method

The experimental TSPDF is straightforwardly obtained as the Fourier transform of the properly corrected powder diffraction pattern for the material [7]. It turns out, from the nature of the scattering equations, that this 1D function is a histogram that gives the probability of finding two atoms in the material sep-
rated by the distance $r$. As such it not only represents a good fingerprint of a material structure, but it is also a very intuitive function, allowing rapid investigation of structures, and changes in structure. For example, the PDF from an organic molecule will have a sharp peak at 1.4 Å, a second neighbor distance at around 2.5 Å and so on. Peaks in the function continue to higher-$r$ values as long as there are well-defined interatomic distances present in the structure up to the size of the crystallite. By seeing where the peaks disappear one can determine immediately, without complicated modeling, an average crystallite size, which is less than or equal to the particle size in the material (it will be less if there is significant structural disorder). It is straightforward to calculate crystallite size in the material (it will be less if there is significant structural disorder). It is straightforward to calculate TSPDFs from structural models [13] allowing a quantitative assessment of material structure. Most importantly in the context of amorphous and nanocrystalline APIs, unlike crystallography, it doesn’t assume a long-range ordered crystalline structure; it may still be used for clumps of packed molecules as small as 1 nm, indeed for studying the molecule itself.

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**Applications of TSPDF in characterization of α- and n-APIs**

Fingerprinting is a critical part of pharmaceutical development and manufacturing. It is desired simply to determine whether a particular form of an API is present in the sample. This is an important safety, quality control and intellectual property (IP) protection issue. It is also important in studies of drug stability, since pharmaceuticals may change their solid form with time. It is also important in studies of drug stability, since pharmaceuticals may change their solid form with time. In another interesting development, it was recently shown that pair distribution functions of a quality sufficient for semiquantitative analysis of nanostructure in materials could be obtained from rather standard configuration transmission electron microscopes.

A frequent question is whether it is possible to detect dilute quantities of an API in a formulation or, for example, in suspension in a solvent. It is difficult to detect small amounts of material, and a *de facto* lower limit of a few percent was considered a good rule of thumb for being able to detect dilute species [7]. However, using the latest data reduction techniques and very intense x-ray beams, it was recently demonstrated that nanoparticles of an API could be detected at the level of 0.25 wt% in aqueous solvent [15], a surprising level of sensitivity, especially given the weak scattering from the organic, but a very exciting development.

Beyond fingerprinting and phase quantification it would be wonderful to be able to extract quantitative structural parameters from α- and n-drugs by structural modeling. This has been done in inorganic materials for some time [7] but challenges exist when transferring this to molecular systems, for example, differentiating intramolecular atomic-pairs and moving atoms in mutually rigid parts of a molecule as rigid bodies. Progress has made in both areas [16] [Frill D et al. Solution and refinement of organic crystal structures by fitting to the atomic pair distribution function (PDF), 2015]. There is still much work to do in making these methods easy to use and robust, but the initial signs are promising. For example, in [16] it was possible actually to solve the (already known) structure of a rigid molecule, quinacridone, using only PDF data.

**Experimental determination of TSPDFs**

The first reported TSPDFs from pharmaceuticals were obtained using intense synchrotron radiation [9]. The high fluxes of high energy x-rays available at such sources make them very attractive for TSPDF studies [12]. Access to synchrotron sources is surprisingly easy and affordable for both academics and industrial users and should not be overlooked as a possibility, often with mail-in programs becoming available meaning that researchers don’t even have to travel to the beamline for data to be collected. However, data suitable for PDF analysis may also be obtained from laboratory x-ray sources [12], but PDFs of sufficient quality for reliable fingerprinting (and further analysis) do require short wavelength x-rays as obtained from silver or molybdenum x-ray tubes.

In another interesting development, it was recently shown that PDFs of a quality sufficient for semiquantitative analysis of nanostructure in materials could be obtained from rather standard configuration transmission electron microscopes [17]. When working with molecular materials, beam damage is an issue. However, it was recently shown that with careful data collection protocols, these approaches may be extended to pharmaceuticals and small molecule systems [18].
Finally, we note that anything that you can do with PDFs, such as fingerprinting, quantification and structural analysis, can now be done in a spatially resolved manner with resolutions down to the micron scale, with the marriage of computed tomography and PDF [19]. This very recent development has not been applied in the pharmaceutical area, but would allow the quantification of nanoparticle structure and morphology of an n-API component, to be mapped out spatially, for example, in a pill compress. The experiments are non-destructive and map nanostructure inside solid objects since the short-wavelength x-rays penetrate into the bulk.

**Future perspective**

Motivated by the desire to characterize α- and n-pharmaceuticals, a great deal of development has recently gone into TSPDF methods and we now have exceptionally powerful tools available for studying drugs and formulations at the nanoscale. But these tools have applications well beyond amorphous drugs, as they can be used to study the structure of any nanoparticle, *ex situ or in situ*. As the nanobio community becomes more aware of these methods, we expect to see many exciting applications to studies that were hardly dreamed of at the beginning of this journey.

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