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Editorial

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Electron diffraction: Accelerating drug development

Electron Diffraction (ED) is gaining momentum in science and industry. The application of ED for performing nanocrystallography is a disruptive innovation that is opening up fascinating new perspectives particularly for organic compounds required in the fields of chemical, pharmaceutical and advanced materials research.

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The three-dimensional structure is the information that is key in order to unambiguously determine the identity, chemical nature and function of a drug molecule. While the structure formula provides general information about the functional groups and the intramolecular connectivity, the 3D structure describes atom positions, bonding and conformation. These details can be crucial to be able to identify interaction sites and thus to understand or predict the reactivity and functionality of a molecule. Moreover, the 3D information is fundamental in the case of chiral molecules, as usually only one enantiomer is bioactive and beneficial, while the other has lower activity and/or higher toxicity.

Molecular structure and crystal structure

For active pharmaceutical ingredients (APIs), besides the elucidation or confirmation of the molecular structure, a great deal of time and effort is invested in the determination of the crystalline solid forms. Because the majority of pharmaceuticals are administered in solid form, the crystal structure is especially important information. The intermolecular packing interactions in the solid state indeed influence multiple physicochemical properties (e.g. stability, melting point, dissolution rates), which in turn affect biological properties (e.g. bioavailability, potency, toxicity) and manufacturability (e.g. tableting properties and shelf life).[1] Considering that the majority of solid APIs are able to crystallize in multiple polymorphic forms, a significant amount of effort is devoted to the early discovery of as many forms as possible and to the assessment of their relative stability, formation and interconversion pathways. Indeed, comprehensive knowledge of the phase diagram allows pharmaceutical companies to identify the solid form(s) that are most suitable from the therapeutical and manufacturing point of view. Furthermore, this knowledge makes it possible to secure a broader protection of intellectual property.

Nowadays, the investigation of solid forms is being even further expanded to explore not only polymorphs but also hydrates, solvates, salts, co-crystals and amorphous forms of a given API. This approach, referred to as crystal engineering, is aimed at achieving an even better control of the properties of solid APIs or formulations.

Besides the detailed characterization of APIs, there are several other stages in the process of drug development at which it is

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desirable or necessary to determine the 3D molecular structure. The structure of intermediate products or biproducts at any step of organic synthesis can facilitate the understanding of the reaction mechanism and possibly guide the optimization of the synthetic pathway. Moreover, such fundamental characterization can greatly support pharmacodynamics and pharmacokinetics studies. The structure of a drug candidate interacting with its therapeutical target (e.g. protein, nucleic acid, etc.) provides valuable evidence of the activity, potential efficacy and mechanism of interaction. On the other hand, the structure of drug metabolites can help elucidate the biotransformation pathways of APIs.

Structure determination today

Structural characterization of pharmaceutical molecules is usually carried out with X-ray diffraction (XRD). In particular, single-crystal XRD is the preferred method for obtaining an accurate and unambiguous 3D model and, notably, the absolute configuration of chiral molecules. However, it is only suitable for those compounds that are able to crystallize and to form crystals large enough for obtaining measurable diffraction data. Finding the conditions to grow crystals of suitable size and quality can be a tedious process, requiring a significant investment of time, resources and expertise. Microcrystalline powders can be analysed by powder XRD, which is a high-throughput and non-destructive technique that enables the identification and also quantification of known phases in mixtures. However, the power of this technique for the ab-initio determination of unknown crystalline and molecular structures is limited. The interpretation of powder XRD pattern can be extremely challenging, especially in case of low crystal symmetry, high molecular weight, large unit cells, and in the presence of crystalline contaminants. XRD-based crystallographic methods require a few milligrams of – ideally – phase-pure crystalline products, and this requirement may be hard to meet in the early stages of drug development. Other interesting compounds, such as reaction intermediates, biproducts, metabolites or natural products, are often available only in limited amounts and purity. Some non-conventional methods for synthesis, screening of solid forms or purification (e.g. mechanochemistry, deep-eutectic solutions, supercritical CO_2) often yield exclusively micro- or nanocrystalline products.

When the compounds do not form crystalline solids or single crystals, the structure can still be investigated via nuclear magnetic resonance (NMR), mass spectrometry (MS) and spectroscopic methods. These methods provide indirect information on the 3D molecular structure, rather than on the crystal structure, and are best used in a complementary fashion.

The future of structure determination

The characterization of the molecular and crystal structure, despite its key importance, can act as a bottleneck in drug discovery. The gold-standard method, single-crystal XRD, is limited by the crystal size. The aforementioned restrictions of XRD can be overcome by using electron diffraction (ED) for structural determination.[2] ED is a crystallographic technique like XRD, and as such it retains the power to unambiguously determine the 3D molecular and crystal structure down to atomic resolution, including the absolute configuration. The strong interaction between electrons and matter (~1000 times stronger compared to x-rays), and the ability to obtain parallel electron beams with diameters well below 1 μm, make it possible to collect measurable diffraction data even from single micro- or nanocrystals (down to a few tens of nanometers). By contiguously sampling a large portion of space (similarly to single-crystal XRD), ED delivers diffraction data suitable for ab-initio structure solution. Several variations of this technique have been developed in less than 15 years,[3,4] and ED has been the key to solving long-standing crystallographic problems for many types of solid materials, including pharmaceutical molecules, biological macromolecules and protein-drug complexes. The structures can be solved and refined by applying the same methods and software that are well-established for X-ray crystallography.[5]

Recent technological and methodological advances have made it possible to mitigate some intrinsic shortcomings of the technique, thus laying the foundations for ED to finally see a breakthrough.[3] The strong interaction of the electron beam with the crystal is indeed the cause of well-known disadvantages. First of all, many organic samples are sensitive to electron-beam damage, which can induce amorphization or decomposition in a matter of minutes or seconds. This can be now overcome thanks to improved data-collection procedures and new detectors based on hybrid pixel or CMOS technology, with a high frame rate and low (or zero) readout noise. Diffraction data with a sufficient signal-to-noise ratio can be acquired using a very-low-intensity incident beam and short exposure times, thus minimizing the beam damage on the crystal. Secondly, for many decades it was believed that multiple diffraction and inelastic scattering would alter the diffracted intensity data to such an extent that they would become unusable for solving crystal structures. Nevertheless, over the recent years, some strategies for data collection and analysis have proved useful to minimize these contributions and to collect quasi-kinematical ED intensity data, which are suitable for structure solution and refinement with conventional methods.

This remarkable evolution has triggered another important stride: the appearance of dedicated instrumentation developed and optimized only for electron diffraction experiments. This in turn is suggesting that ED will soon become widely available as a complementary tool in crystallographic or analytical laboratories. Until today, performing ED experiments required adapting transmission electron microscopes for this purpose as well as specialized expertise, so that the lack of ease-of-use has been so far an effective deterrent for the adoption of ED within the pharmaceutical industry and research.

Beyond structure determination

ED is now an attractive solution for supporting drug discovery and drug development. By overcoming the need to grow large crystals, the characterization of crystal structures will become a routine analysis, powerfully accelerating the discovery of new drug candidates and their transition from the bench to the market, as researchers will gain more insight into synthetic processes, solid form screenings, formulations and drug-target interactions.[6–8]

Using an electron beam for crystallographic analysis opens a series of new possibilities and unique advantages. Electron beams can be easily shaped to diameters of 1 μm or less, making it possible to analyze the individual crystals in a mixture of phases or polycrystalline aggregate, thus mapping the various components. A similar approach can be applied to study formulations and, for example, detect submicrometric crystalline nuclei in amorphous solid dispersion.

Moreover, thanks to the computational power of modern computers, the dynamical theory of diffraction can be taken into account for the refinement of crystal structures, making it possible to reach a higher level of accuracy and finer details of the structural model. Remarkably, this includes crucial information, such as the accurate position of H atoms and the unambiguous determination of the absolute configuration.[9,10]

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