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Combining electron diffraction and calorimetry to find new polytypes of molecular compounds of pharmaceutical interest

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Summary. — This article proposes a new method integrating thermal and electron diffraction analyses for the detection and characterization of new polytypes of pharmaceutical compounds. In the first part, the importance of polymorphism in the pharmaceutical science will be discussed, then the main features of the experimental techniques used will be described, and finally, after looking at some selected examples, a proposed experimental protocol will be exposed.

1. – Introduction

Molecular pharmaceuticals are of utmost importance, being of great relevance both for human health and for basic science investigations. Our attention will be focused on two phenomena, crystallization and polymorphism, which, despite decades of systematic studies, are still highly debated both from the basic science viewpoint, due to the lack of a unitary theory, and for pharmaceutical applications, playing a pivotal role in drug formulation and stability.

In pharmaceutical science there are two key parameters that govern the adoption of a given compound [1]: the bioavailability, namely the compound fraction that arrives at the targeted destination, and the dissolution rate, *i.e.*, the speed at which a compound dissolves in a specific medium. It is a well-known fact that the physical state influences these two parameters [2]. The crystalline state is usually preferred for practical applications, being the thermodynamically most stable state. Nevertheless, for a specific molecule, the crystalline structure is not necessarily unique because the same molecule can pack in different configurations, each with a different free energy. This phenomenon is called *polymorphism*. Different crystalline structures are accompanied by

different bioavailability and dissolution rate [3], but the physical stability of a polytype upon storage, *i.e.*, over time and temperature, is not always guaranteed [4]: hence the necessity of a quality control process called *polymorph screening*. A strict relation exists between the micro- and macroscopic regimes of a generic compound, *i.e.*, between its crystalline structure and its thermodynamic behaviour, and both aspects have a great impact on the physico-chemical properties of the final product. Hence having a clear picture of both aspects is of crucial importance to understand the possible interactions of the molecule and its possible applications. The different polytypes of a specific molecule, if present, can be prepared in several manner, *i.e.*, chemically, mechanically or thermally. In this article, sample preparation via thermal methods will be considered.

2. – Experimental techniques

Differential Scanning Calorimetry (DSC) is a well established technique for materials thermal characterization widely employed because it requires only a small amount of sample (up to tens of milligrams) and a short experimental time (a complete analysis may require from few minutes to a couple of hours). Thanks to the possibility to perform heating and cooling ramps at constant rate, it is possible to directly link the measured signal, the *heat flow*, to the specific heat capacity of the sample. The heat flow permits to track the physical status of the sample, *e.g.*, crystalline or amorphous, and to access the related thermodynamical quantities, like enthalpy, entropy and Gibbs free energy. Finally, by performing more sophisticated time-temperature ramps, it is possible to monitor the kinetic evolution of the desired process, like the crystallization from the supercooled liquid at constant temperature.

3D Electron Diffraction (3DED) is a fundamental and emerging technique for obtaining the crystalline structure of micro- and nano-crystalline materials. It has undergone significant development in the last 20 years thanks to improvements in data collection strategies, in single electron detectors, and in the reduction of the electron dose on the sample, permitting to obtain reliable crystalline structures even from organic materials, despite their beam sensitivity. Moreover, it is now possible to acquire data from nanocrystals with size too small even for the large-scale X-rays facility [3]: indeed, the strong interaction of electrons with matter, permits to study crystals down to 10–100 nm. Finally, due to the ease of extracting and focusing electrons in beams with a diameter of 10 nm (or even less), it is possible to spatially resolve a specific specimen, and to check if more than one crystalline domain is present in the same specimen [3].

3. – Experimental results

Throughout literature, 3DED has shown its extraordinary capabilities in solving crystalline structures. One of the first notable cases is Orthocetamol [5], which had been known for more than one century without anyone succeeded in growing a crystal big enough for X-ray synchrotron investigations. Thanks to 3DED it was possible to solve its structure for the first time. More recently, ϵ-Vemurafenib showed the same difficulty and its crystalline structure was solved with 3DED [6].

Another interesting case were 3DED demonstrated its exceptional potential is the case of Olanzapine Form III [7]. In fact, Olanzapine Form III cannot be obtained in a pure form, but only in combination with the Form II. Thanks to the spatial selectivity across the sample granted by the small beam diameter, it was possible to isolate a region were only Form III exists and to solve its structure.

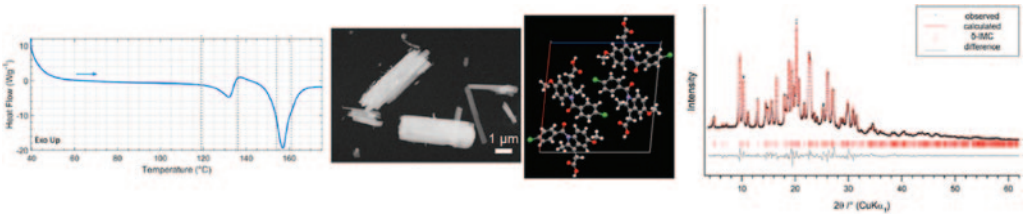

Fig. 1. – Schematic application of the proposed method. From left to right: DSC measurement to ensure presence of polymorphs; selection by STEM imaging of suitable crystal for 3DED; crystalline structure obtained from 3DED experiment; validation of the obtained structure against PXRD data. Panels are taken from ref. [5].

On the other hand, employing DSC it is possible to gain an insight into the thermodynamic behaviour of a generic compound. As demonstrated in the case of L-Arabitol [4], even though a method exists to produce a new polytype, reverse to the most stable form can happen during storage, *i.e.*, by keeping the sample at constant temperature for a prolonged time. Thus, with DSC it is not only possible to obtain information on the crystallization process itself, but also on the physical stability of the specific crystalline form. In addition, an extensive analysis can enable to produce a phase diagram of the polytypes to understand which is the most stable form throughout a certain temperature interval [4].

Summarizing, the combination of 3DED and DSC can bring results of high interest for basic science and for the pharmaceutical industry. In the final part of the paper, a possible workflow on how to integrate DSC and 3DED will be exposed.

As a first step, a thermal characterization to ensure the presence of polytypes is needed. If polytypes are present, then some stability checks must be performed: a possible test consists in keeping the polymorphic form at constant temperature (similar to the storage one) for a significant time (*e.g.*, a couple of days). This step is needed to monitor the physical stability of the crystal in time, because in case of high metastability there will be a reverse to the most stable form. Then, the produced sample must be placed in the electron microscope for 3DED measurements. To do so, the micro-sample holder used for the DSC measurements must be opened, and the powder put on the 3DED grid. Note that the amount of sample produced in a DSC experiment (usually units of milligrams) is more than enough for 3DED experiments, where only some hundreds micrograms are required. Finally, a validation of the obtained structure against a standard is welcome. Thus, a powder X-rays diffraction (PXRD) experiments is needed and the 3DED model must be compared against the PXRD data via a Rietveld refinement. If it is impossible to perform a PXRD measurement, it is appropriate to proceed with a dynamical refinement of the obtained 3DED crystal structure [8]. A schematic representation, with expected outcomes, is reported in fig. 1.

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