Electron Diffraction Tomography/Micro-ED for Structural Characterization of Pharmaceutical Compounds

NanoMEGAS
Advanced Tools for electron diffraction

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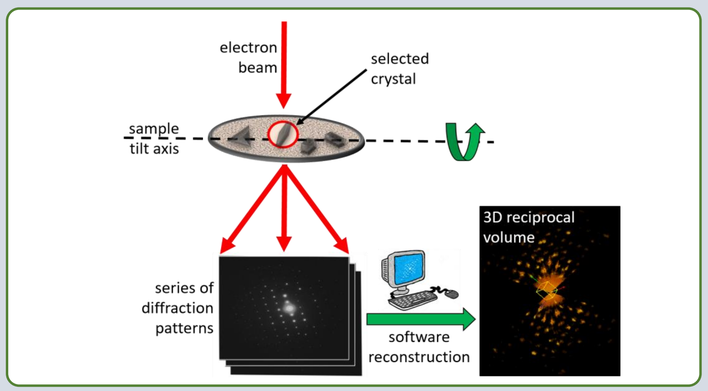
Introduction

In recent years, the scientific community has shown a renewed interest in use of 3D Electron Diffraction (3D-ED)/Micro Electron Diffraction (Micro-ED) for characterization of pharmaceutical compounds. For many API's (active pharmaceutical ingredient), it is always challenging to grow suitable size crystals for single crystal X-ray diffraction. Powder X-ray Diffraction (PXRD) has its own challenges e.g. (a) low crystallinity of the sample, which produces a broadening of peak profiles (b) long cell parameters and pseudo symmetries, which lead to peak overlap even at low and medium resolution (c) presence of minor impurities or polymorphic forms. In all those cases, 3D-ED/Micro-ED in Transmission Electron Microscope (TEM) could be a useful alternative for structural studies, as crystals as small as 50 nm can be studied.

Principle of 3D ED data collection in TEM

The principle of acquiring 3D-ED data consists on focusing the electron beam on a nm size crystal in TEM/STEM mode and sampling the reciprocal space in small steps (usually 1 degree tilt or less) using beam precession or using continuous rotation (Micro-ED with or without beam precession) of the crystal.

As organic crystals are often very beam sensitive, data collection can be done either at room temperature and/or at cryo-conditions using pixel-ated detectors at low dose conditions (< 0.01e/Å^2/ sec) at STEM mode. The acquired 3D-ED data can be processed to determine *ab-initio* unit cell, space group, atomic positions and moreover, hydrogen atom positions can also be determined [1].



References

[1] M. Gemmi, E. Mugnaioli, T. E. Gorelik, U. Kolb, L. Palatinus, P. Boullay, S. Hovmöller, J. P. Abrahams. ACS Central Science **2019**, 5(8), 1315-1329.
[2] E. van Genderen, M.T.B. Clabbers, P. Pratim Das, I. Nederlof, A. Stewart, Q. Portillo, S. Nicolopoulos, J.P. Abrahams, Acta Crystallogr. A **2016**, 72, 236–242.
[3] P. P. Das, E. Mugnaioli, S. Nicolopoulos, C. Tossi, M. Gemmi, A. Galanis, G. Borodi, M. M. Pop, Org. Process Res. Dev. **2018**, 22, 1365-1372.
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Case Studies 1: Carbamazepine Structure

Carbamazapine (CBZ) is primarily used in the treatment of epilepsy and neuropathic pain. It may be used in schizophrenia along with other medications and as a second-line agent in bipolar disorder. CBZ exists in several polymorphic forms.

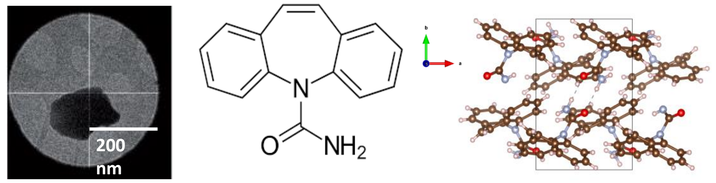

Figure 1. TEM Image of the carbamazepine nanocrystal and ab-initio solved structure from 3D-ED data at RT [2].

Experimental 3D-ED Unit Cell (RT)
a = 7.68 Å
b = 11.44 Å
c = 13.92 Å
β = 91.22°
SPG: P21/n

Literature Reported (SCXRD-RT)
a = 7.534 Å
b = 11.150 Å
c = 13.917 Å
β = 92.94°
SPG: P21/n

Case Studies 2: Ramelteon Structure

Ramelteon is the first selective melatonin MT1 and MT2 receptor agonist approved by the U.S. Food Drug and Administration (FDA) in 2005 for the treatment of insomnia.

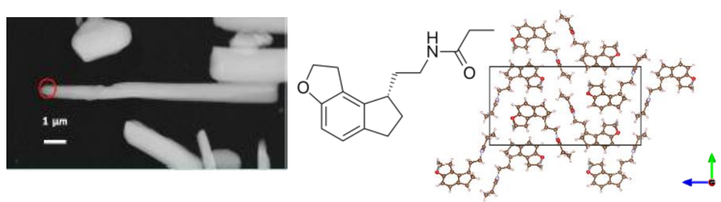

Figure 2. TEM Image of the Ramelteon crystals and solved structure from 3D-ED data at RT by Simulated Annealing [3].

Experimental 3D-ED Unit Cell (RT)
a = 4.99 Å
b = 11.59 Å
c = 22.95 Å
SPG: $P2_12_12_1$

Unit cell from SCXRD study (RT)
a = 5.0450 (4) Å
b = 12.4178 (11) Å
c = 23.187 (2) Å
SPG: $P2_12_12_1$

Case Studies 3: Loratadine Form II Structure

Loratadine, available under the brand name Claritin among others, is a medication used to treat allergies. Loratadine exists in two different polymorphic forms.

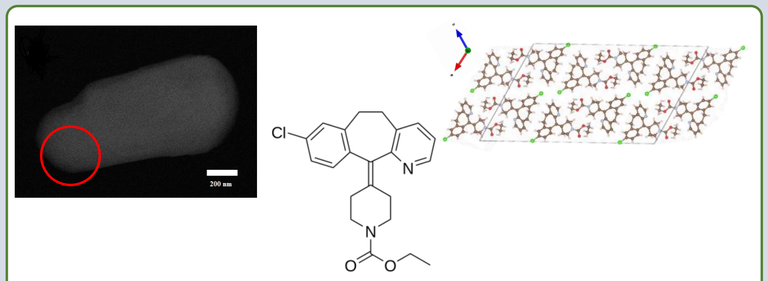

Figure 3. TEM Image of the metastable Loratadine Form II crystal and solved structure from 3D-ED data at RT by Simulated Annealing [4]

Experimental 3D-ED Unit Cell (RT)
a = 35.41 Å
b = 5.28 Å
c = 22.56 Å
β = 118.21°
SPG: C2/c

Literature Reported Unit cell (SCXRD-LT)
a = 35.652(10) Å
b = 5.206(2) Å
c = 22.743(6) Å
β = 117.418(14)°
SPG: C2/c

Case Studies 4: Linagliptin Structure

Linagliptin, sold under the brand name Tradjenta among others, is a medication used to treat diabetes mellitus type 2. As of now 30 polymorphs of Linagliptin are known to exist. Our current structure matches closely with one of the patented form of which no structure was previously reported.

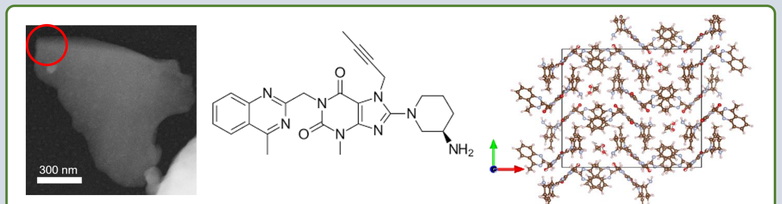

Figure 4. TEM Image of the Linagliptin crystal and ab-initio solved structure from 3D-ED data at RT [5]

Experimental 3D-ED Unit Cell (RT)
a = 24.85 Å
b = 21.57 Å
c = 9.74 Å
SPG: $P2_12_12$

Unit Cell from Synchrotron data (RT)
a = 24.85091(13) Å
b = 21.56916(9) Å
c = 9.74376(4) Å
SPG: $P2_12_12$

Conclusions

Our results show that 3D-ED/Micro-ED techniques in combination with Direct Detection cameras can be used as a powerful tool for phase identification and structural characterization for nm size (50-500 nm) beam sensitive pharmaceutical materials.