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Experiment Report

Proposal HC-5404
"The structure of pharmaceuticals subjected to cryo-milling and compression"
Beamline: ID22
Beam time allocated: 6 shifts
Beam time used: 6 shifts
Start-finish date: 16/02/2024-18/02/2024
Beamline scientist: Andrew Fitch

The aim of the experiment was to study the atomic-scale and local intermolecular structure of active pharmaceutical ingredients (APIs) produced in the amorphous-like states using various methods such as vitrification, quenching, pressure-densification, and cryomilling. The non-crystalline forms of pharmaceuticals are characterized by much better solubility in water than their crystalline counterparts. Therefore, they have potential for uses in pharmacy and medicine. However, their structural properties and physical stability may differ, depending on the preparation method. For example, recently we showed that cryo-milled phase of itraconazole is much more physically stable (it does not recrystallize for a longer time) than its vitrified form while the pressure-densified glass is even more stable than the cryo-milled phase. This behavior needs explanation on the atomic-scale level. For our studies we used APIs differing in the molecular structure and able to form various supramolecular clusters or mesophases and exhibiting an organization of molecules on the medium-range scale. We wanted to probe the intra- and intermolecular structure of the selected APIs using the X-ray diffraction combined with pair-distribution function method. The X-ray diffraction data in the reciprocal space may fingerprint in more detail the medium-range order while the pair distribution function in the real space is a useful tool to provide details of the local nearest-neighbour structure and intramolecular structure as well.

ESRF was necessary for this experiment since ID22 beamline enables diffraction measurements with a very high energy (we used around 70 keV) that allows for collecting data in a wide range of scattering vectors up to around 29 $Å^{-1}$ and obtaining the pair distribution function with a good resolution in the real space that is crucial for studying the amorphous-like APIs. First we try to collect data using high-resolution 9-channel Si 111 multianalyser. However, the samples very quickly experienced radiation damage. Therefore, finally, the data were collected using 2D medical Perkin-Elmer detector. The first day, for each studied sample/phase we collected 100 scans, each lasting 4 minutes. On the second day, in order to enhance the statistics, we collected 150 scans for each probed sample phase. The measurements were performed at ambient conditions, for as-received samples (some of them

were prepared one day before the experiment in the chemical laboratory) as well as on heating mode up to temperatures of around 200°C using an electric heater. Automatic sample changer – a robot was employed for the first night shift. Unfortunately, on the morning of the second day, there was a problem with the computer controlling the measurements and we were able to run the next portion of samples only in the evening of the second day. Luckily, we could extend the data collection up to Sunday afternoon.

The collected diffraction data were calibrated using LaB6 reference, reduced using pyFAI software, and transferred to the form of one-dimensional function of the diffracted intensity versus the scattering angle 2θ. An example of parameters used for measurements and data reduction is presented below:

```
# pyfai_version = 2023.5.0
# ewoks_version = 0.3.0
# xunits = 2th_deg
# application = pyfai-integrate
# version = 3
# do_mask = True
# mask_file = fabio:///data/visitor/hc5404/id22/20240216/PROCESSED_DATA/processing_2D/ITR-V_HRBS/hc5404_mask_HR2.edf
# do_dark = False
# do_flat = False
# do_polarization = True
# polarization_factor = 1.0
# do_dummy = False
# do_2D = False
# nbpt_rad = 4900
# unit = 2th_deg
# do_radial_range = True
# radial_range_min = 0.0
# radial_range_max = 49.0
# do_azimuthal_range = True
# azimuth_range_min = 125.0
# azimuth_range_max = 145.0
# chi_discontinuity_at_0 = False
# do_solid_angle = False
# method = ['*', '*', '*']
# error_model = poisson
# dist = 0.38000039
# poni1 = 0.042910722077313446
# poni2 = 0.4085080746660507
# rot1 = 0.0024334534489669868
# rot2 = 0.005851225373872582
# rot3 = 0.0
# detector = Perkin
# detector_config.pixel1 = 0.0001
# detector_config.pixel2 = 0.0001
# monitor_value = 6636178.0
# reference_value = 50000.0
# energy = 7.000407488361119590e+01 keV
# wavelength = 1.771099734398839547e-11 m
```

Below is the list of samples measured in the experiment, all the substances were listed in the sample A form – to study in this experiment session:

1) Itaconazole (ITR) – vitrified
2) Itraconazole (ITR) – crystalline
3) Itraconazole (ITR) – quenched
4) Itraconazole (ITR) – pressurized
5) Itraconazole (ITR) – cryomilled
6) Itraconazole (ITR) – melted
7) Fenoprofen calcium dihydrate (FENCA) - crystalline
8) Fenoprofen calcium dihydrate (FENCA) – vitrified
9) Fenoprofen calcium dihydrate (FENCA) – cryomilled
10) Ritonavir (RIT) – crystalline
11) Ritonavir (RIT) – vitrified
12) Ritonavir (RIT) – cryomilled
13) Ritonavir (RIT) – pressurized
14) Ritonavir (RIT) – quenched
15) Ritonavir (RIT) – melted
16) Ritonavir (RIT) – vitrified heated at 40°C
17) Ritonavir (RIT) – cryomilled heated at 40°C
18) Ritonavir (RIT) – pressurized heated at 40°C
19) Ritonavir (RIT) – quenched heated at 40°C
20) Loratadine (LOR) – crystalline
21) Loratadine (LOR) – vitrified
22) Loratadine (LOR) – cryomilled
23) Loratadine (LOR) – pressurized
24) Loratadine (LOR) – quenched
25) Loratadine (LOR) – melted
26) Empagliflozine (EMP) – crystalline
27) Empagliflozine (EMP) – vitrified
28) Empagliflozine (EMP) – quenched
29) Empagliflozine (EMP) – cryomilled
30) Empagliflozine (EMP) – vitrified aged
31) Empagliflozine (EMP) – cryomilled aged
32) Empagliflozine (EMP) – melted
33) Empagliflozine (EMP) – vitrified heated at 60°C
34) Empagliflozine (EMP) – quenched heated at 60°C
35) Empagliflozine (EMP) – cryomilled heated at 60°C
36) Empagliflozine (EMP) – vitrified aged heated at 60°C
37) Empagliflozine (EMP) – cryomilled aged heated at 60°C
38) Sulfapyridine (SUL) – crystalline
39) Sulfapyridine (SUL) – melted
40) Sulfapyridine (SUL) – quenched
41) Sulfapyridine (SUL) – vitrified
42) Sildenafil (SIL) – crystalline
43) Sildenafil (SIL) – melted
44) Sildenafil (SIL) – vitrified
45) Sildenafil (SIL) – vitrified aged
46) Sildenafil (SIL) – quenched
47) Sildenafil (SIL) – cryomilled
48) Lopinavir (LOP) – crystalline

49) Lopinavir (LOP) – melted
50) Lopinavir (LOP) – vitrified
51) Lopinavir (LOP) – quenched
52) Lopinavir (LOP) – cryomilled
53) Aripiprazole (ARP) Sigma – crystalline
54) Aripiprazole (ARP) Sigma – vitrified
55) Aripiprazole (ARP) TCI – crystalline
56) Aripiprazole (ARP) TCI – vitrified
57) Aripiprazole (ARP) TCI – cryomilled
58) Empty capillary for background
59) Si NIST 640e reference for instrumental resolution

The selected diffraction data collected during the experiment are shown below:

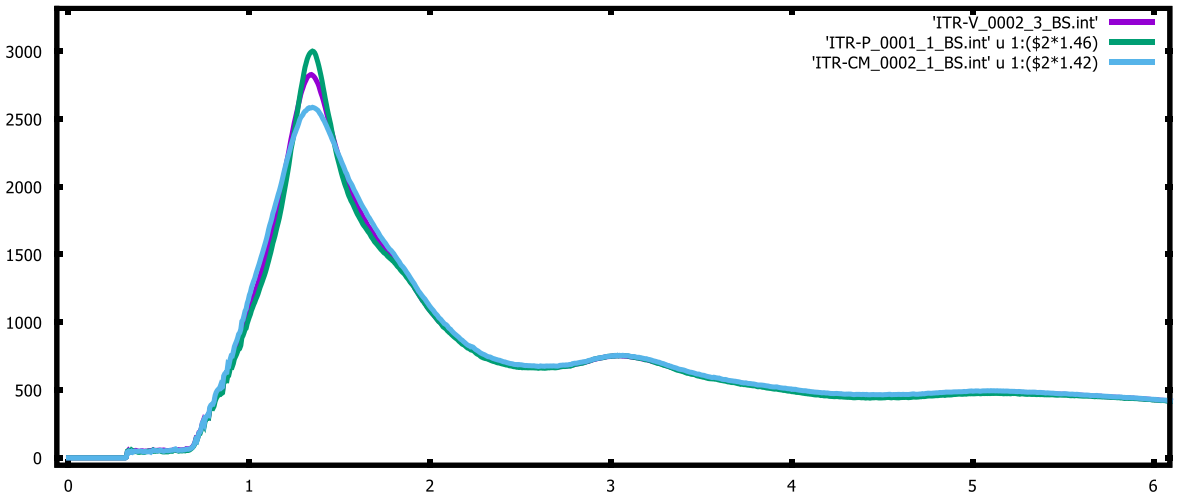

Fig. 1. The obtained diffraction data for different phases of itraconazole (ITR) produced using vitrification (V), pressure-densification (P) and cryomilling (CM).

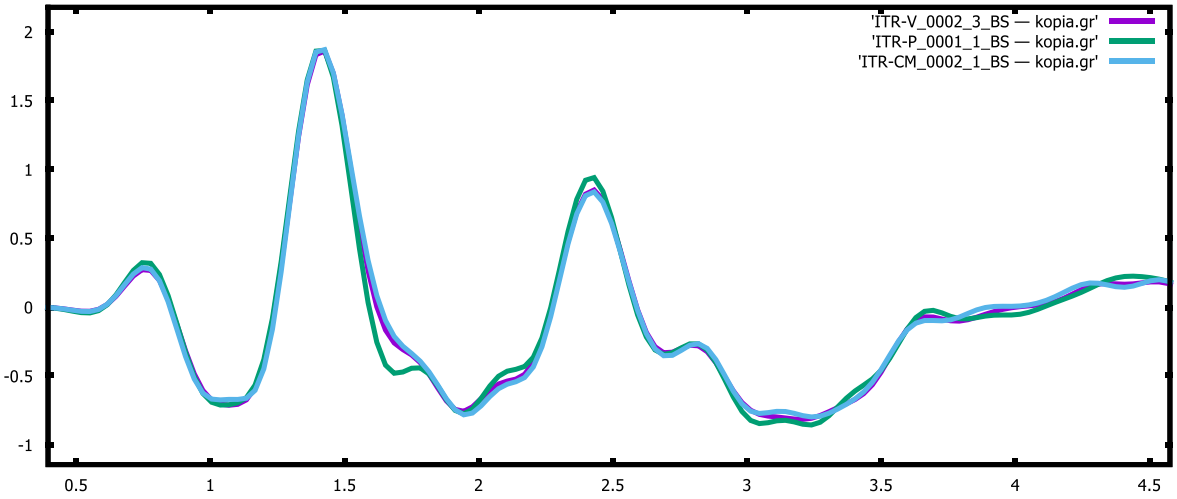

Fig. 2. The computed atomic pair distribution function based on the collected diffraction data for different phases of itraconazole (ITR) produced using vitrification (V), pressure-densification (P) and cryomilling (CM).

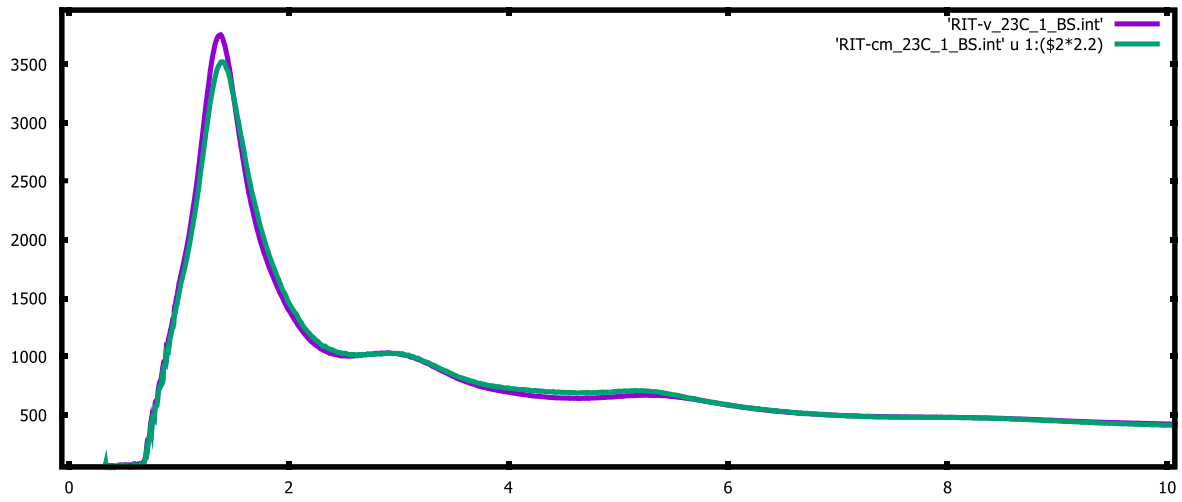

Fig. 3. The obtained diffraction data for different phases of ritonavir (RIT) produced using vitrification (v) and cryomilling (cm).

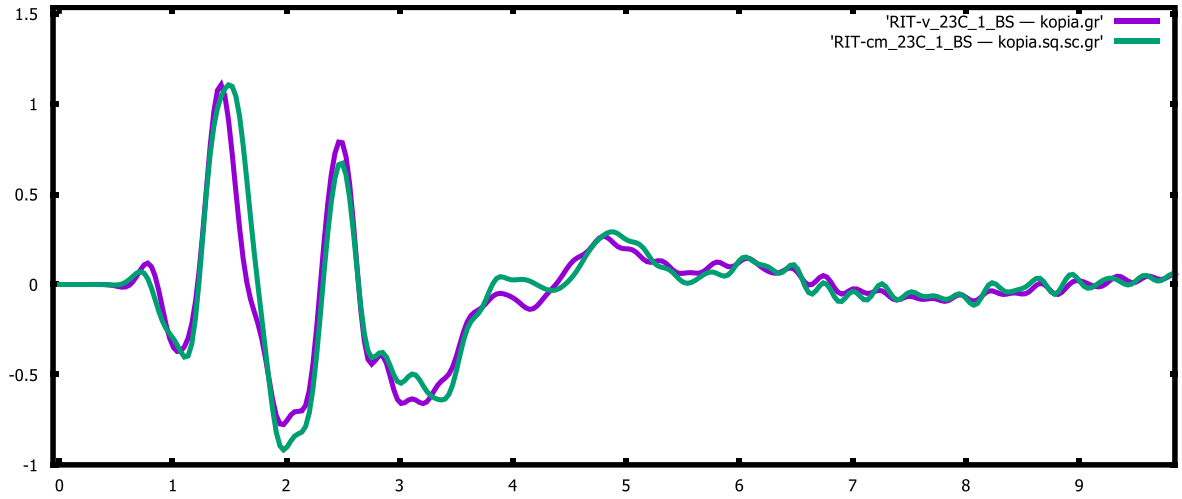

Fig. 4. The computed atomic pair distribution function based on the collected diffraction data for different phases of ritonavir (RIT) produced using vitrification (v) and cryomilling (cm).

The obtained data will be analyzed in terms of the properties of the main diffraction peak and the pre-peaks at the low scattering vector range. The pre-peaks are fingerprints of the medium range ordering of the molecules. Moreover, the data will be used to compute and analyse the pair distribution functions. The obtained results will be set together with the data of dielectric spectroscopy measured at the University of Silesia as well as with the results of differential scanning calorimetry, Raman and infrared sepectroscopy, and other complementary techniques. They will be used to understand the structure of amorphous phases of pharmaceuticals fabricated using various methods

We hope, the results of this experiment will be published in journals with good impact factors.