EXPERIMENT 5

X-RAY POWDER DIFFRACTION (XRPD)

Determination of crystallinity in indomethacin

LEARNING AIMS

- To gain familiarity with the use of XRPD equipment
- To understand the applications and limitations of XRPD
- To gain understanding of the interpretation of X-ray diffractograms
- To extend data manipulation and presentational skills
- To continue developing GLP skills

LEARNING OUTCOMES

- To critically discuss the use of XRPD in pharmaceutical analyses
- To manipulate XRPD instrumentation
- To enable the differentiation of amorphous from crystalline phases
- To learn methods of quantification in XRPD

DIRECTED READING

OMED 0104 – LECTURE NOTES

Cullity, B.D. 2001. *Elements of X-ray Diffraction*. Prentice Hall. 555pp.

Whiston, 1987. C. *X-ray Methods*. Analytical Chemistry by Open Learning. John Whiley. Chapters 1-3.

Storey, R. & Royall, P. 2011. *Solid state characterization of pharmaceuticals*. Wiley-Blackwell. Chapter 2.

INTRODUCTION

X-Ray Diffraction is used in pharmaceutical analyses particularly in the fields of crystal structure determination. Areas of interest include phase identification, determination of degree of crystallinity, estimates of crystallite shape and size, quantitative analysis of mixtures, determination of polymorphic state.

X-ray Diffractometers consist of an X-ray generator, water-cooling system, an X-ray tube, a collimation system, slits, sample changer, monochromator, X-ray detector, a two-circle goniometer. Data are acquired via an interface to pc and are manipulated using software.

Experimental considerations include selection of: an X-ray tube with suitable anode, X-ray generator setting (kV and mA), slit widths, step scanning speed, scanning range in degrees two-theta, suitable sample preparation.

This equipment produces ionizing radiation and as such falls under the remit of the Ionising Radiations Regulations 2017. All procedures must be carried out in accordance with the Local Rules which are attached to the equipment.

EXPERIMENTAL

Due to the safety requirements of the Local Rules much of the experimental work in this practical will be carried out as a laboratory demonstration. Therefore, it is essential that adequate notes are taken while the equipment and methods are being demonstrated.

Determination of crystallinity in indomethacin

Material: one sample of pure crystalline indomethacin, one sample of pure amorphous indomethacin, one sample of a mixture of crystalline and amorphous indomethacin.

To achieve good data samples need to be ground to a particle size of 5-10 microns; there should be an even particle size distribution within the sample. Ground samples are packed into the plastic sample holder being aware not to introduce any preferred orientation effects, as this causes incorrect intensity ratios of the diffraction events; this becomes important when quantification is required. Beware of altering the crystal structure by grinding, sometimes it may be necessary to use cryogenic grinding.

Diffractograms are collected using suitable scan parameters. Referring to the ICDD PDF pattern for indomethacin we can see that most of the diffraction events for indomethacin fall between 10.2 and 37.4$^{O}2\theta$, so for the two end member forms, scan parameters are chosen to encompass these values i.e. to run from 2 to 50 $^{O}2\theta$. An intermediate size of exit slit is selected (0.6mm) to give a good compromise between signal and peak shape resolution. Using a fast position sensitive detector with an acquisition time of 0.1 second and a step size of 0.04 degrees will take 1 minute and 30 seconds to collect the data. Better quality data with less noise and a higher signal can be obtained by increasing the counting time, for example to 0.2 or 0.3 seconds per step; the data acquisition time will increase accordingly.

For the sample containing a mixture of crystalline and amorphous indomethacin it is necessary to acquire top quality data for the purposes of Rietveld refinement. Therefore, a smaller exit slit can be used (0.2 mm) to improve peak shape, and a longer counting time is used (0.3 seconds per step) to improve signal to noise ratio.

EVALUATION OF RESULTS

Qualitative assessment using the software (Bruker, EVA v6.0):

1) import the data for pure crystalline and pure amorphous forms into EVA.
2) overlay the two diffractograms: crystalline and amorphous, note the differences
3) using the crystalline form, perform a peak search and generate a list of d-spacings and intensities, export to Excel for output. This is for comparison to the ICDD data below.
4) perform a background subtraction in readiness for the search-match procedure.
5) perform a search-match routine to confirm the identity of the phase: examine the ICDD card and compare with your data, note any differences and think about why they may have arisen.

Quantitative assessment using the software (Profex):

6) import the mixture data into Profex
7) run the Rietveld refinement and integrate the diffractogram to measure the total counts under the curve (crystalline + amorphous)
8) note the measured areas
9) subtract the amorphous background from the total area to leave crystalline area only
10) calculate the percentage crystallinity by ratio. = (net area of the crystalline component / net area for the total area (crystalline + amorphous)) * 100

Data for qualitative assessment - ICDD data file for indomethacin

triclinic P1, a=9.348Å, b=11.006Å, c=9.764Å, α=69.3, β=110.88, γ=92.76

Angle 2-Theta °	d value Angstrom	Intensity	Angle 2-Theta °	d value Angstrom	Intensity
10.160	8.699	22	29.335	3.042	31
11.599	7.623	100	30.407	2.937	14
12.734	6.946	15	33.545	2.669	10
16.650	5.320	54	34.112	2.626	11
17.004	5.210	89	34.765	2.578	8
17.281	5.127	25	37.415	2.402	13
18.557	4.778	19			
19.280	4.600	36			
19.599	4.526	60			
20.289	4.374	10			
20.862	4.255	15			
21.795	4.074	89			
22.890	3.882	17			
23.136	3.841	15			
24.001	3.705	22			
25.459	3.496	13			
26.584	3.350	42			
27.437	3.248	15			
28.273	3.154	11			
28.879	3.089	23			

QUESTIONS

1. What is the effect on the diffractogram of changing slit sizes?
2. What is the purpose of water-cooling, and why is it necessary?
3. Explain what the effect of a non-random powder sample would be on the diffractogram?
4. What is the effect on peak shape of crystallite size?
5. How is the n-term in the Bragg equation seen in diffractograms?
6. Why do we use monochromatic radiation as a source?
7. How can you explain intensity ratios in your samples which differ from the ICDD?
8. Which terms in the Bragg equation are the instrumental parameters?
9. What would changing the scan speed achieve, and why may you want to do this?
10. Why does a diffractogram of an amorphous phase differ from a crystalline phase?