3RD YEAR ADVANCED PRACTICALS: CRYSTALLOGRAPHY
POLYMORPHISM IN THE SOLID STATE

Background

Crystallography is the area of science associated with the solid state, specifically crystalline materials and their properties, ie factors like structure, packing and relative orientation of molecules and their impact on melting point, solubility, bioavailability etc.

Solids have greater diversity in their structure due to their static positions in comparison to liquids and gases where molecules have random distribution due to thermal motion. The structures of molecular compounds in the solid state are dependent upon the forces between the component molecules. These forces hold molecules together in a lattice and give the molecular arrangement and positions relative to other molecules and relative to the crystal as a whole.

This experiment will give you an insight into standard crystallographic methods (single crystal and powder) for examining structure and standard techniques for characterisation and analysis of the solid state. You will learn how these different techniques interlink and may be used together to give the complete evaluation of a solid compound. You will look at polymorphism in crystals – a highly important area in the field as different polymorphs have different physical properties and can be detrimental, for example, in pharmaceutical drug discovery. A drug candidate will be a specific polymorph carried through the discovery, development, testing, regulatory stages etc. A new polymorph can, generally unintentionally, form at any stage due to only the smallest of changes in these processes. The new polymorphic form will have different properties and may not have the therapeutic, or other, effects required. Polymorphism control and understanding the phenomena is important in many industries and areas of science.

Polymorphism in molecular crystallography occurs when a solid chemical compound exists in more than one crystalline form. The forms differ in their physical properties and may also have differing chemical properties. Packing polymorphs have a different packing arrangement within the crystal structure while conformational polymorphs contain a different conformer of the compound (different conformation through rotation of bonds).

Polymorphs of a compound will generally have different relative stabilities in different environments and be kinetically or thermodynamically favoured leading to the ease and conditions of forming.

There are many factors affecting polymorphism, some of which include:

- Repetition of the experiment
- Solution history
- Humidity
- Dryness of solvents/solute
- pH or trace acid/alkalinity in organic solvents
- Impurities in the solvent/solute
- Scale of the process
- Container material and surface
- Agitation
- Seeding (accidental or intentional)
- Temperature of nucleation
- Nucleation inducing effects, eg Temp
- Viscosity of solution
- Polarity of solution
- Concentration
- External pressure
- Temperature/Cooling regime
- Time of storage of crystallised solution

- Anti-solvent / solvent
- Operator

Aims

To demonstrate the ability to control the solid form; the conditions of crystallisation will affect the way molecules pack into a solid state structure and how they interact with each other. The conditions can be altered through a variety of possible ways including varying the solvent (the simplest) and alternative techniques such as vapour diffusion, temperature regimes (heating/cooling), evaporation, sublimation, additives and rates of heating/cooling/evaporation.

Through crystallisation techniques, produce two polymorphs of a pharmaceutical-like compound and characterise these using single crystal and powder diffraction techniques. Solve the crystal structure and gain an understanding of the very acute geometric information that single crystal diffraction can provide. Use various analytical techniques to support the formation of two polymorphs and the differences between the structures.

Introductory Reading Material:

The following Oxford Chemistry Primer books are all available as short term loans from the library and serve as *basic introductory text only for the characterisations techniques you are going to use*. An understanding of the chemistry of these systems can only be gained from the published literature.

1) Polymorphism & crystallisation in 'From Molecules to Crystallizers' by Roger Davey & John Garside; ISBN: 978-0198504894
2) Principles of Single Crystal X-ray Diffraction in 'Crystal Structure Determination' by William Clegg; ISBN: 978-0198559016
3) Description of Powder X-ray Diffraction within 'Inorganic Materials Chemistry' by Mark Weller; ISBN: 978-0198557982

Before the practical we recommend that you look through previous lecture course notes on the area and into crystallisation techniques as you will be needing to make some informed decisions in this area during the practical.

Safety

As you progress and become more independent in your work and research it is important you are responsible for the safety of each task or experiment you carry out. You will be expected to have an understanding of the substances to be used in the practical and to complete a COSHH form for the work before starting. This should be done prior to the practical and brought for inspection at the beginning of the first day.

Experiment Overview

The experiment will predominantly involve the recrystallisation of glycine (sourced from chemical suppliers) using different conditions to recrystallise two different polymorphs. In addition there will be an aspect of literature searching to familiarise yourself with the area and available literature on the topic. There is a vast array of literature and you will be required to try to find and distinguish the most relevant and appropriate articles. You will be recording your observations as you perform the experiment in an *Electronic Laboratory Notebook* (LabTrove) – this is an industry standard approach.

Once crystals have been grown successfully, characterisation and analytical techniques will be used to quantitatively distinguish between the two polymorphs and understand the relationship of the two structures. It is important to use the various analyses in conjunction with one another to build up a picture of the structures and properties as a whole, rather than just collecting individual pieces of standalone data or facts. These techniques will include single crystal x-ray diffraction (SCXRD), powder x-ray diffraction (XRPD), hot stage microscopy (HSM), infrared spectroscopy (IR) and differential scanning calorimetry (DSC).

SCXRD

Single crystal diffraction, through several steps and automated calculations, produces a crystal structure, which gives a 3D representation of the compound. Also the technique unambiguously shows connectivity and the relationship in space between both atoms in a molecule and different molecules within the crystal lattice. A dataset for each polymorph will be collected using a single crystal from each recrystallisation carried out. The data set collected will then be worked up using specific software packages to give a 3D structure for each polymorph. The differences between the two structures can be seen using overlays and packing functions in the software package 'Mercury'.

XRPD

XRPD is used to fingerprint solid samples and will give a unique pattern for each of the polymorphs. Additionally patterns can give information on unit cell dimensions and also characterise crystalline samples. The peaks seen correspond to a certain reflection originating from a set of miller indices which relate to the unit cell. For fingerprint characterisation, it is the position (2θ position) opposed to the intensity which is important. Intensity can be dependent upon preferred orientation therefore it is important merely whether a peak at a certain position is present or absent. A few points:

- d-spacings of lattice planes depend upon the size of the unit cell and give the position of the peaks observed.
- Peak intensity results from crystallographic structure ie atom positions in the unit cell and their thermal vibration.
- Line width and peak shape is dependent upon the conditions of the measurement and properties of the sample material (eg particle size).

A homogeneous sample of fine particles is required for this technique (a powder) and therefore some of the crystals grown must be ground up using a pestle and mortar [N.B. ensure a single crystal is left for SCXRD!]. A powder pattern will be collected for each of the polymorphs and also of the starting material to show the differences between the three structures.

HSM

Hot stage microscopy is used to probe the effect of heating a sample up and viewing any physical changes which occur. The temperature and heating rate can be carefully controlled to give an insight into the behaviour of samples through manipulation of the set up. HSM will be used to investigate the two polymorphs and the observations when each is heated up. This should allow the melting point and relative stability of each polymorph to be determined and visual data obtained.

IR

IR is not a technique that is new to you, however it can be used in more ways than those familiar to you from the teaching labs (using the IR spectrum to identify key functional groups within the structure and assign the relevant bands to them). Multiple spectra can be overlaid to compare and contrast, showing differences and similarities between two or more spectra. These observations can be related to the environment of certain atoms due relationship and effect an atom has on another, hence giving variations in vibration of bonds and interactions.

DSC

Differential Scanning Calorimetry is a thermo-analytical technique showing the temperature flow of a sample compared to a reference. The technique is carried out by heating the sample at a controlled rate (eg 10°C/min) and recording the energy required for the heating process. Certain processes will give out or take in heat (exo/endothermic) and therefore more or less heat will need to flow to it to maintain the sample at the same temperature as a reference. DSC measures the heat absorbed or released during such processes hence they will therefore show up on the curve (Wg^{-1} plotted against time/temperature) as peaks or troughs, eg melting, phase transitions, recrystallisations etc.

Tasks to be completed

As part of the practical you will be expected to think for yourselves and use your time efficiently. Below are detailed the tasks you are required to complete for this practical and it is up to you to ensure they are all completed in time. Note that the equipment will be shared between up to 4 pairs and so you should allow for this, as not everyone can do the same thing at the same time due to equipment and space restrictions.

Task	**Group size**	**Data to collect**	**Approximate time to complete**	**LabTrove Template**
Crystallisation	Pair	N/A, note method and observations	~30 mins per polymorph	Recrystallisation Experiment
XRPD	Pair	3x XRPD pattern (two polymorphs and starting material)	5 mins run time per sample plus prep	X-Ray Powder Diffraction 1: Sample Prep X-Ray Powder Diffraction 2: Collecting a powder pattern
XRPD analysis	Individual	N/A – interpret data collected	Any time – can do out of lab time	X-Ray Powder Diffraction 3: Analysing the pattern
SCXRD data collection	Pair	2x single crystal diffraction patterns (one per polymorph)	45-60 mins per data collection	SCXRD 1: Preparing the Crystal SCXRD 2: Running the sample SCXRD 3: Processing the data
SCXRD solve/refine	Pair	Produce .cif file	2-3 hours	SCXRD 4: Solving the Structure
SCXRD analysis	Individual	Use data from before	Any time – can do out of lab time	SCXRD 4: Solving the Structure Using Mercury
HSM	Pair	2x video and observations noted (one per polymorph)	~30 mins per sample plus hot stage cooling and prep time	Hot Stage Microscopy 1: Running the Experiment Hot Stage Microscopy 2: Recording your experiment
IR analysis	Individual	N/A – interpret data given	Any time – can do out of lab time	IR 1: Introduction IR 2: Data IR 3: Analysis
DSC analysis	Individual	N/A – interpret data given	Any time – can do out of lab time	DSC
Literature searching	Individual	Key papers and note any interesting information, findings and references	Any time – can do out of lab time	Literature Searching

LabTrove

You will be recording your experiment using an electronic lab notebook which can be accessed via: http://ugchem.labtrove.soton.ac.uk (note you must be on a university computer or logged into the VPN to access this site). To log in, use your normal university username and password. See additional document in Blackboard for details.

Suggested organisation of time

Day 1 AM

- You will be split into pairs. 2 pairs can do '1' while the others do '2' then swap over once the hot plates lab space is free:

  1 Crystallisation experiments (in pairs, 2 pairs at a time)

  2 Literature searching

- The crystallisation is the most time-critical part of the experiment – everything else depends on it so you should aim to get this done as efficiently as possible. XRPD of starting material can also be performed at this time and possibly one pair will be able to do SCXRD.

Day 1 PM

- Run analysis (HSM/XRPD/SCXRD) if any crystals have formed (get the recryst done as early as possible to have maximum chance of crystals growing by the afternoon).
- Literature searching/look at data given to you to analyse.
- Keep lab notebook up to date with procedures, observation and notes throughout the day.

Day 2

- Run all remaining analyses (noting how much time should be allowed for each data collection) ensuring all data required is collected. Particularly crucial is the collection of the SCXRD data on the second polymorph that will have crystallised during the intervening week, but you will also have to perform HSM and XRPD on this new sample.
- Structure solution and refinement of single crystal data.
- Continue literature searching.
- Look at given data (DSC/IR) for analysing.
- Ensure e lab notebook kept up to date throughout.
- Check you have all data and resources needed.
- 'Hand in' e lab notebook before you leave at the end of the day (note every entry is time and date stamped so it is clear when the entries were made and updated).

Assessment

The assessment of this practical will be in done predominantly through the electronic lab notebook (submitted on leaving the lab on the last day of the practical) and answering given questions (pdf on Blackboard) submitted the following week (9am Wednesday) via Blackboard. These will enable the assessment of your practical skills in the area of crystallography.

An assessment for the whole module is conducted on the practical you are undertaking in Week 7/8. So if it is this practical, you will be required to prepare and submit a presentation and ChemComm review style paper. See course overview and outlines on Blackboard for details and also refer to LabTrove template 'ChemComm Article (week 7/8)'.