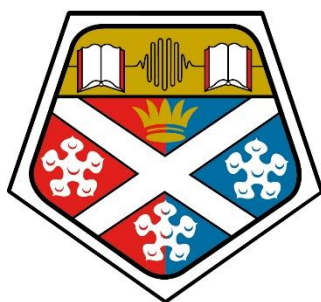




University of
Strathclyde
Glasgow

**From crystal to tablet: structural insights
into the compression behaviour of
pharmaceutical solids**

Strathclyde Institute of Pharmacy and Biomedical Sciences

*A thesis presented in fulfilment of the requirements for the degree of Doctor of
Philosophy in the Faculty of Science of the University of Strathclyde*

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June 2026

Declaration of Author's Rights

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Contributions

Chapters 3 and 5 are based on previously published work. Full bibliographic details of the associated publications are provided on the first page of each chapter.

For Chapter 3, I contributed to data curation, formal analysis, investigation, methodology, validation, visualisation, and writing (original draft and review & editing).

For Chapter 5, I contributed to the preparation of samples, acquisition of data (both at synchrotron facilities and in-house), interpretation of the data, investigation, visualisation, and writing (original draft and review & editing). Specialist expertise in the collection, curation and processing of XRD-CT data was provided by Stephen Price. Electron diffraction data collection and structure solution were carried out by ELDICO Scientific AG.

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Abstract

This doctoral thesis advances the understanding of how pharmaceutical solids respond to applied pressure, from the molecular to the macroscopic scale, with relevance to the tableting process.

The compression of racemic ofloxacin and chiral forms γ -levofloxacin and levofloxacin hemihydrate was followed using single-crystal and powder X-ray diffraction across hydrostatic and non-hydrostatic conditions, with Raman spectroscopy used to confirm a solid-form change in one case. All three were shown to be soft, highly compressible solids dominated by dispersive π -stacking, with the most compressible direction perpendicular to the molecular stacks. Each structure compressed smoothly through the tableting pressure range (< 0.3 GPa) but underwent distinct phase transitions at higher pressures; the transitions depended on hydration, particle size and pressure-transmitting medium.

Subtle pressure-driven molecular rearrangements can be overlooked by conventional high-pressure data analyses. A new method and accompanying Python tool, MolDis, were developed to calculate and visualise pressure-induced molecular displacement relative to a reference molecule directly from variable-pressure diffraction data. MolDis was validated against paracetamol Forms I and II and applied to a set of acetanilides (4-chloroacetanilide, the monoclinic form of 4-methylacetanilide, metacetamol Form I), revealing the flattening of corrugated chains and layers and displacement normal to potential slip planes, complementing interaction-energy, void and slip-plane analyses.

Lastly, X-ray Diffraction Computed Tomography was applied to intact pharmaceutical tablets, using glycolide as a pressure-sensitive marker. This non-destructive approach spatially resolved a non-uniform Form I to Form II transformation and, on storage, captured an unexpected hydrolysis to a previously unreported phase whose structure was solved by electron diffraction.

Together, these studies follow the structural response to pressure across length scales, contributing to the understanding needed to anticipate mechanical behaviour and solid form change during pharmaceutical manufacturing.

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Abbreviations

AFM	Atomic force microscopy
API	Active pharmaceutical ingredient
CCDC	Cambridge Crystallographic Data Centre
CSD	Cambridge Structural Database
CSP	Crystal structure prediction
DAC	Diamond anvil cell
DESY	Deutsches Elektronen-Synchrotron
DLS	Diamond Light Source
ED	Electron diffraction
EoS	Equation of state
ESRF	European Synchrotron Radiation Facility
FC-70	Fluorinert FC-70 (pressure-transmitting medium)
HAA	2-(2-hydroxyacetoxy)acetic acid
LH	Levofloxacin hemihydrate
Lγ	γ -levofloxacin
MCC	Microcrystalline cellulose
me	Methanol-ethanol mixture (pressure-transmitting medium)
MgSt	Magnesium stearate
O	Ofloxacin
OSD	Oral solid dosage
PCA	Principal component analysis
PDB	Protein Data Bank
pet	Petroleum ether (pressure-transmitting medium)
PTM	Pressure-transmitting medium
SCC	Sapphire capillary cell
SC-XRD	Single-crystal X-ray diffraction
STEM	Scanning transmission electron microscopy
THz-TDS	Terahertz time-domain spectroscopy
XRD-CT	X-ray diffraction computed tomography
XRPD	X-ray powder diffraction

Chapter 1 - Introduction

1.1. Synopsis

This introduction presents the theory and literature that provide context for the principal aim of this thesis. It begins with polymorphism and the range of solid forms adopted by active pharmaceutical ingredients, and the importance of controlling them in the manufacture of oral solid dosage forms. It then narrows to the pharmaceutical processes that induce polymorphic transformation and, in particular, to tableting. The methods used to investigate pressure-driven structural behaviour are reviewed at both the molecular and macroscopic scales, before the differences between high-pressure studies and tableting are brought together to frame the work that follows. Experimental procedures specific to each study are provided within the relevant results chapter.

1.2. Polymorphism in the pharmaceutical industry

The solid form of APIs is particularly important in the pharmaceutical industry for two reasons: APIs are widely formulated as solids in oral solid dosage (OSD) forms; and polymorphism. Most APIs are delivered orally, and tablets are among the most popular OSD forms. Their widespread use reflects several advantages: they allow accurate dosing; they are physically and chemically stable under ambient storage conditions; they can be manufactured cheaply at large scale while offering versatility in drug release; and they are convenient and safe to administer, supporting patient adherence¹.

Most APIs are isolated and delivered as crystalline solids. A crystalline solid forms when molecules adopt a repeating arrangement with long-range periodic order. Molecules in a crystalline solid form may take different molecular conformations and three-dimensional arrangements while maintaining the same chemical identity. When at least two different arrangements are possible, this phenomenon is known as polymorphism, as defined by Halebian and McCrone in 1969² (Figure 1.1). Polymorphism is particularly important in the pharmaceutical industry because APIs can exhibit multiple polymorphs^{3,4}. The different molecular arrangements among polymorphs produce distinct networks of intermolecular interactions, which give rise to different physical and physicochemical properties that directly influence product performance, stability and manufacturability^{5,6}. As a result, identifying and controlling polymorphism is a central aspect of drug development, and extensive solid-form screening is conducted to identify the most suitable polymorph for manufacturing and patient delivery^{7,8}.

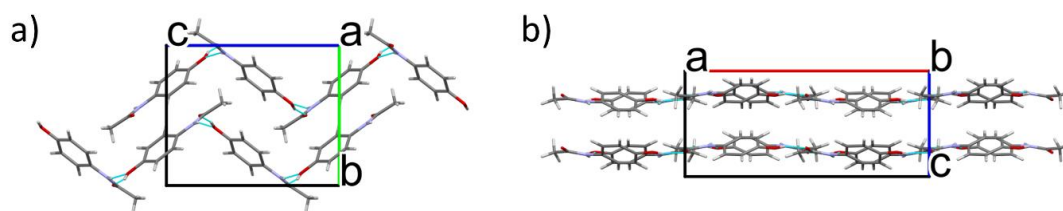


Figure 1.1. (a) Paracetamol Form I; (b) Paracetamol Form II.

Beyond polymorphs, APIs may exist in a range of solid forms⁹, including:

- Co-crystals: crystalline form containing an API and one or more different molecules in a stoichiometric ratio;
- Solvates: crystalline form containing an API and an organic solvent in a stoichiometric ratio;
- Hydrates: crystalline form containing an API and water in a stoichiometric ratio;
- Salts: crystalline form containing an ionic species of an API and a counter-ion;
- Amorphous phases: structure lacking long-range periodic order.

Each form has different properties, similar to polymorphs, hence the need to also identify the most suitable form for manufacturing and patient needs⁷. Different solid forms can themselves be polymorphic, provided their chemical composition is maintained; a compound can also exist in distinct amorphous states, a debated phenomenon known as polyamorphism. For hydrochlorothiazide, for instance, distinct amorphous forms have been reported to exhibit different physical and thermal stabilities¹⁰.

1.3. Pharmaceutical processes inducing polymorphic transformations

The crystal structure of an API is sensitive to external conditions such as temperature and pressure and may change solid-state form as a result of the stimuli¹¹. Because both heat and mechanical pressure are present during the processing steps of pharmaceutical tablet manufacture, understanding how these factors induce polymorphic transformations carries practical value. A robust manufacturing process can be designed either to avoid such transformations or to induce them deliberately by identifying the conditions under which they consistently occur¹².

Pressure-induced polymorphic transformations are well documented in pharmaceutical systems^{13,14} with examples reported for a range of APIs during processing, as discussed below. Milling and compaction apply external pressure to an API during tablet manufacturing^{15,16} and the way in which pressure is applied shapes the outcome. In a pharmaceutical processing context, pressure is described in terms of stress, which is the force per unit area within a material and may act either normal to a surface or parallel to it (i.e. shear stress). Processes involving shear, such as milling and tableting, can drive polymorphic transformation whereas the application of uniform (hydrostatic) pressure is generally less effective under comparable conditions. For chlorpropamide, the A and C polymorphs interconvert during compaction at pressures as low as *ca.* 10.5 MPa^{17,18}, but not under hydrostatic compression (11.7 MPa), applied above the compaction conversion threshold, indicating that shear stress rather than pressure alone drives the transformation through the activation of a slip system¹⁸, a concept described in Section 1.5.2.1.

Milling has been observed to induce polymorphic transformation and amorphisation. Real-time synchrotron X-ray diffraction during the ball-milling of D-mannitol captured a direct conversion from the β to the α polymorph with no detectable amorphous intermediate, attributed to the close structural similarity between the two forms¹⁹. High-shear cryogenic milling causes the amorphisation of crystalline sucrose²⁰. More broadly, the growing use of mechanochemistry has highlighted the susceptibility of many organic compounds to structural change under mechanical activation, including when a small amount of solvent is present (i.e., liquid-assisted-grinding)²¹.

During tablet compaction, the powder blend is typically subjected to pressures of up to *ca.* 300 MPa (0.3 GPa)²², though studies of compaction-induced transformations often extend beyond this. Conversion of chlorpropamide form C to form A is observed at the radial tablet surface at pressures as low as 25 MPa²³, and caffeine Form I converts to Form II through a transient intermediate form that relaxes to Form II over several days, a change detectable from pressures as low as 5 MPa²⁴. Compaction can also alter crystallinity: partial amorphisation has been reported in posaconazole between 50 and 400 MPa²⁵, and in theophylline and amlodipine besylate from 100 MPa and nitrofurantoin from 250 MPa (each studied up to 1000 MPa)²⁶, while amorphous celecoxib has been shown to devitrify to Form III from 27.4 MPa upwards²⁷.

In addition to these molecular-level changes, the mechanical properties and macroscopic behaviour of an API under stress are central to developing an effective drug product formulation and manufacturing process. For example, a material's resistance to deformation influences how it behaves during processing and the quality

of the final tablet^{1,28}. The next section examines the tableting process and how materials respond to the stresses involved.

1.4. The tableting process and the mechanical response of pharmaceutical materials

The tableting process transforms a powder into a coherent compact through applied pressure. Prior to compaction, the API is blended with excipients (i.e., inactive materials in a formulation) to obtain a homogeneous powder blend, which is then confined within a die and subjected to a uniaxial force, applied through a punch, to form a tablet²⁹.

Tableting is a complex and heterogeneous process, with multiple mechanical mechanisms acting simultaneously and non-uniformly within the powder blend. As uniaxial force is applied, the powder bed densifies, reducing porosity and increasing interparticle contact. The early stages are dominated by particle rearrangement as particles occupy void space to achieve a more efficient packing. As the void space diminishes, the stress at interparticle contact points rises and leads to particle deformation and/or fragmentation depending on the material's mechanical properties. Deformation can be elastic, recovering once the stress is removed, or plastic, producing a permanent change. These mechanisms drive further densification and develop the interparticulate bonds that give the tablet its mechanical strength³⁰.

The mechanical behaviour of a material is characterised by its mechanical properties, such as elastic modulus, hardness, yield stress and bulk modulus, which quantify the relationship between stress (defined in Section 1.3) and strain (i.e., the

deformation that results from it)^{31,32}. In the context of compaction, the performance of a powder is commonly described using three related concepts:

- Compressibility: the ability of a powder to decrease in volume during compaction;
- Compactability: the ability of a powder to form a mechanically strong compact;
- Tabletability: the relationship between compaction pressure and the resulting tablet strength.

Together, these properties provide a quantitative basis for comparing the mechanical performance of different solid forms³⁰.

The mechanical response of an API is not uniform across the tablet. Because force is applied uniaxially by the punch, it is transmitted unevenly through the powder bed and lost to friction between particles and the die wall and the punches, generating normal and shear stresses (Figure 1.2). The stress within the tablet is therefore anisotropic, varying in magnitude with direction, and produces density gradients across the tablet with regions near the surface experiencing higher stress than in the interior²³. The gradients lead to spatial variations in the microstructure and can alter the API's solid state^{23,33}.

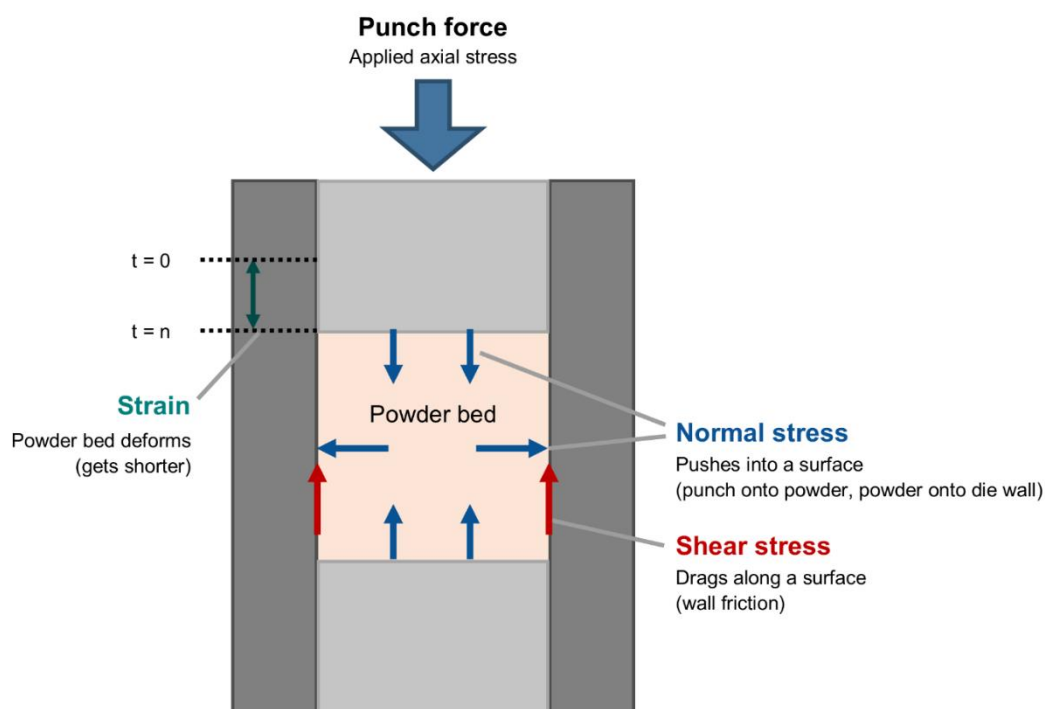


Figure 1.2. Labelled schematic of the stress and strain in a powder bed during uniaxial tablet compaction.

Pressure-driven changes are therefore molecular in origin and unevenly distributed within the tablet. This is illustrated by the systems introduced in Section 1.3. When compacted on their own, the polymorphic transformation of chlorpropamide from Form C to Form A²³ and the amorphisation of posaconazole²⁵ have both been shown to concentrate at the tablet surface rather than at the core, where die-wall friction generates the greatest shear stress. These solid-state changes follow the heterogeneous stress distribution generated during compaction, connecting the mechanical environment described in this section to the phase transformations introduced in Section 1.3. Methods used to characterise these changes and to understand how materials respond to pressure are described in the next section.

1.5. Characterisation methods of pressure-driven solid-state behaviour

Understanding pressure-induced structural changes in APIs requires techniques that probe behaviour across multiple length scales. At the molecular level, diffraction and spectroscopic methods provide insight into the crystal structure, molecular packing and intermolecular interactions. At the macroscopic level, mechanical and imaging techniques reveal deformation behaviour and how structural change is distributed within a tablet, while crystallographic analysis links this behaviour to the molecular structure. No single technique captures all aspects of pressure-driven behaviour, and a more complete understanding relies on combining complementary approaches.

1.5.1. At a molecular level

Experimental investigation of pressure-induced structural change at the molecular level is primarily carried out using diffraction and spectroscopic techniques. These approaches rely on the interaction between matter and radiation and provide complementary information on how crystalline APIs respond to applied pressure.

1.5.1.1. Crystalline solids

A crystalline solid consists of a molecular arrangement that is repeated periodically in three dimensions (Figure 1.3). The periodicity is described by a lattice, which is an infinite array of points with identical surroundings. The smallest repeating unit is the unit cell, a parallelepiped defined by three edge lengths (a , b , c) and three angles (α , β , γ); translating the unit cell along the lattice directions generates a crystal structure³⁴. Within the unit cell, the smallest structurally unique portion is called the asymmetric

unit, from which the remaining cell content is generated by symmetry operations (Figure 1.3a-c). Depending on the symmetry relations, crystals are grouped into seven crystal systems, ranging from low symmetry triclinic to high symmetry cubic. Organic APIs most commonly crystallise in low-symmetry systems such as triclinic and monoclinic³⁵. This feature becomes important when describing their anisotropic response to pressure.

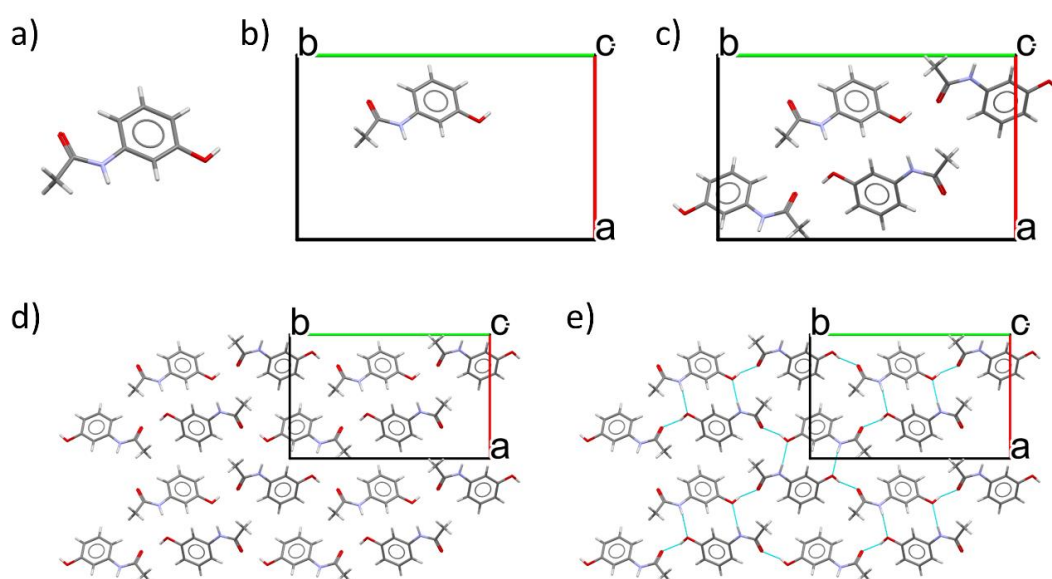


Figure 1.3. (a) Single metacetamol molecule; (b) asymmetric unit for metacetamol Form I; (c) unit cell of metacetamol Form I; (d) 2x2x2 expanded unit cell of metacetamol Form I; (e) 2x2x2 expanded unit cell of metacetamol Form I showing hydrogen bonds in the crystal structure.

1.5.1.2. Diffraction

Diffraction is the primary method for determining the atomic-scale structure in crystalline materials. It resolves molecular conformation and crystal packing at the Ångström scale, enabling structural changes such as variations in unit cell parameters and intermolecular distances to be monitored as a function of pressure^{13,36}. Diffraction is based on the coherent elastic scattering of radiation, in which incident radiation interacts with the periodic arrangement of atoms in a crystal to produce a diffraction

pattern, from which structural information can be extracted. Three types of radiation are used for diffraction studies: X-rays (i.e., photons), neutrons and electrons³⁷. Depending on the type of radiation used, scattering occurs from different parts of the atom: X-rays are scattered by the electrons of the atoms, neutrons by their nuclei, and electrons by the atomic electrostatic potential. When the path difference between the waves scattered from successive parallel atomic planes satisfies Bragg's law, constructive interference occurs, producing a discrete reflection³⁸ (Figure 1.4). Bragg's law is given by

$$n\lambda = 2d \sin \theta \quad (1)$$

where n is the order of the reflection (an integer), λ is the wavelength of the incident radiation, d is the spacing between adjacent planes and θ is the angle between the incident radiation and the plane. Each reflection can be associated with a specific set of crystallographic planes, described by their Miller indices (hkl). Together, reflection positions and intensities contain the information needed to determine the crystal structure³⁴.

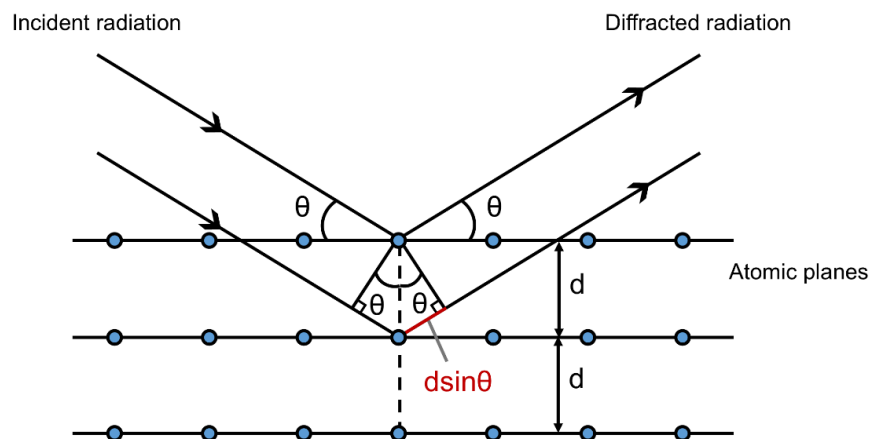


Figure 1.4. Schematic of Bragg's law, showing radiation diffracted from parallel atomic planes spaced a distance d apart at angle ϑ . Constructive interference, producing a reflection, occurs when the path difference between adjacent planes ($2d \sin \vartheta$) equals a whole number of wavelengths.

The positions of the reflections describe the geometry of the unit cell. Assigning Miller indices to each reflection and determining the unit cell from these positions is known as indexing. Once the pattern is indexed, the d-spacing of an (hkl) reflection is used to calculate the unit cell parameters of a sample. The intensities of the reflections reflect the arrangement of atoms within the unit cell. The absence of certain reflections (i.e., zero intensity) indicates specific structural features linked to translational symmetry, either the centring of the lattice or the presence of glide or screw symmetry elements.

The atomic positions, however, cannot be determined directly from reflection intensities because of the way they relate to the structure factors. The structure factor connects the arrangement of atoms in the unit cell to the intensities measured in a diffraction experiment, and is a central parameter linking structure to data. It describes the amplitude and phase of the wave scattered by all atoms, and is given by

$$F(hkl) = \sum_{j=1}^N f_j e^{-2\pi i(hx_j + ky_j + lz_j)} \quad (2)$$

where N is the number of atoms in the unit cell, f_j is the atomic form factor (the scattering power) of the j -th atom, (x_j, y_j, z_j) are the fractional coordinates of the j -th atom in the unit cell, and (hkl) are the reflection's Miller indices. The structure factor can also be expressed as

$$F(hkl) = |F(hkl)| e^{i\phi_{hkl}} \quad (3)$$

where $|F(hkl)|$ is the amplitude and ϕ_{hkl} is the phase. Diffraction detectors record only the intensity of each reflection, which is proportional to the squared amplitude of the structure factor

$$I(hkl) \propto |F(hkl)|^2 \quad (4)$$

Because the amplitude is squared, the phase is lost and cannot be recovered directly. This is known as the phase problem. The phases, however, are required to calculate the electron density (the distribution of electrons throughout the unit cell, whose maxima correspond to atomic positions) by the inverse Fourier transform

$$\rho(x, y, z) = \frac{1}{V} \sum_{hkl} F(hkl) e^{2\pi i(hx + ky + lz)} \quad (5)$$

where $\rho(x, y, z)$ is the electron density and V is the unit cell volume.

For small molecules, the phase problem is commonly solved using direct methods, which use statistical relationships between structure factor amplitudes to estimate a set of phases. The resulting structure is then refined against the experimental data using least-squares minimisation, improving the agreement between the observed and calculated structure factors.

1.5.1.3. X-ray, neutrons and electrons

Of the three radiation types, X-ray diffraction is the most widely used due to its versatility and the widespread availability of lab-based instruments³⁹. Neutron diffraction offers enhanced sensitivity to hydrogen and deuterium atoms, enabling precise determination of their positions within a structure⁴⁰. However, organic materials scatter neutrons weakly, requiring large sample sizes (typically on the order of mm³) and access to a neutron facility³⁹. Electron diffraction, an emerging field, is particularly powerful for determining the crystal structure from very small or thin crystals that are not suitable for conventional X-ray diffraction⁴¹. The strong interaction of electrons with matter allows high-quality data to be obtained from such small volumes, but can cause significant radiation damage in organic solids, which must be carefully managed during data collection.

1.5.1.4. Single-crystal and X-ray powder diffraction

X-ray diffraction measurements can be performed on either a single crystal or a polycrystalline sample, giving rise to single-crystal X-ray diffraction (SC-XRD) and X-ray powder diffraction (XRPD), respectively. In SC-XRD, the diffraction data collected consist of a three-dimensional set of discrete reflection intensities, from which the positions of individual atoms can be determined with high precision⁴² (Figure 1.5a). This makes SC-XRD the most reliable method for crystal structure determination, in which structures are solved using the diffraction data without prior assumptions⁴³.

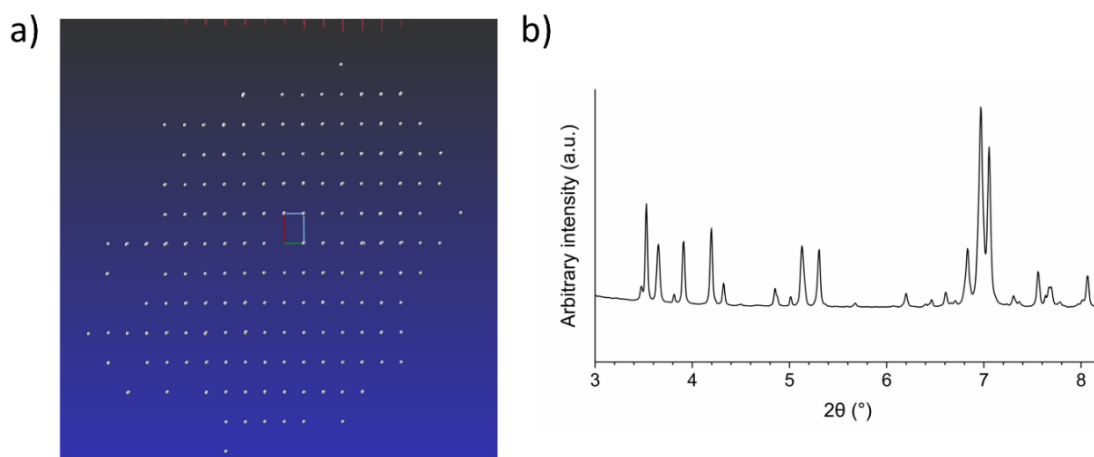


Figure 1.5. Representative X-ray diffraction data. (a) Reciprocal lattice plot from single-crystal X-ray diffraction of 2-dimethylsulfuranylidene-1,3-indanedione (YLID); (b) powder diffraction pattern of levofloxacin hemihydrate, collected at ambient pressure and 30.0 keV ($\lambda = 0.410691 \text{ \AA}$) on beamline ID15B at the European Synchrotron Radiation Facility (ESRF).

In contrast, XRPD is performed on a polycrystalline sample made up of many randomly oriented small crystals. The diffraction intensity is measured as a function of diffraction angle (2θ), resulting in a one-dimensional pattern⁴⁴ (Figure 1.5b). The unit cell can be determined from the peak positions by indexing the pattern. Once the initial unit cell has been obtained, it can be refined against the full pattern using whole-

pattern fitting methods, such as the Pawley or Le Bail method, without requiring a structural model^{45,46}. More detailed analysis can be achieved through full profile refinement (i.e., Rietveld method), in which a structural model is fitted to both the positions and intensities of the diffraction peaks, enabling refinement of atomic positions within the crystal⁴⁷.

1.5.1.5. Synchrotron radiation

In addition to laboratory sources, X-ray diffraction can be performed using synchrotron radiation. A synchrotron produces a far more intense and focused X-ray beam than a laboratory instrument, and its wavelength can be tuned, allowing small or weakly diffracting samples to be measured quickly and at high resolution⁴⁸.

Synchrotron radiation is the electromagnetic radiation emitted when charged particles travelling close to the speed of light are deflected by a magnetic field. Electrons are produced by thermionic emission from a heated cathode in an electron gun and accelerated towards a linear accelerator, in which a sequence of oscillating radiofrequency fields raises their speed to almost that of light. A booster ring then increases their energy into the giga-electronvolt (GeV) range before they are injected into a storage ring under ultra-high vacuum in which the electrons circulate for hours⁴⁹.

At each corner of the storage ring, bending magnets force the electron beam to change direction. Because a change in direction is itself an acceleration, and accelerating charges radiate, electromagnetic radiation is emitted tangentially to the storage ring's orbit. Depending on the energy of the circulating electrons and the magnetic field strength of the bending magnet, different wavelengths can be accessed, from the infrared to hard X-rays. Quadrupole and sextupole magnets keep the

circulating beam focused and on its correct path, while radiofrequency cavities replace the energy the electrons radiate⁴⁹.

In third-generation sources, such as Diamond Light Source (the UK's national synchrotron, operating at 3 GeV), the straight sections between the bending magnets house insertion devices (wigglers and undulators), each an array of alternating magnets that drive the electrons along an oscillating path⁵⁰. With each oscillation, the emitted radiation adds together, producing a beam far more intense and directional than that from a single bending magnet. Wigglers increase the intensity of the radiation, while undulators concentrate the radiation flux into narrow, selectable wavelength bands that are tuneable by adjusting the magnet gap. The resulting brilliance (i.e., the flux per unit source area and beam divergence) exceeds that of laboratory sources by many orders of magnitude⁴⁹. More recently, fourth-generation sources such as the ESRF Extremely Brilliant Source (ESRF-EBS), operating at 6 GeV, have used multi-bend achromat lattices to lower the electron beam emittance (its size and angular spread) further, achieving a substantial gain in brilliance and coherence⁵¹.

The emitted radiation is delivered to a beamline, which refers to the apparatus that conditions and delivers the radiation from the source to an experimental station. It consists of an optics hutch, an experimental hutch and a control cabin. In the optics hutch, the polychromatic beam is tuned for the experiment: a monochromator selects a single wavelength, and the beam is focused by grazing-incidence mirrors. When a smaller beam spot size is needed, compound refractive lenses⁵² or zone plates⁵³ are used to reach micron and sub-micron sizes. The beam then travels to the experimental hutch, which houses the diffractometer and detector. Fast area detectors with continuous readout allow a dataset to be collected in seconds, and the hutch is lead-

shielded and interlocked so that the beam is admitted only once no personnel are inside.

These characteristics make synchrotron radiation well-suited to studying pressure-driven structural change in organic materials, which are weak X-ray scatterers. The high flux produces usable diffraction from small sample volumes. In a diamond anvil cell, described in Section 1.5.1.8, the backing seats and metallic cell body absorb part of the beam and shadow the diffracted X-rays, restricting the range of diffraction angles (2θ) over which data can be collected; selecting a short wavelength (high photon energy) in a synchrotron compresses the diffraction pattern to low 2θ angles so that more of the pattern falls within the opening angle, which is an important advantage for the completeness of high-pressure data, particularly for single-crystal measurements⁵⁴. Additionally, the low divergence, also called collimation, of the beam produces sharp, well-separated reflections, which help in the indexing of large or low-symmetry organic unit cells, the deconvolution of overlapping powder peaks, and the resolution of weak reflections against the background.

X-ray diffraction computed tomography, introduced in Section 1.5.2.3, also relies on high flux, small beam size and rapid readout. Reconstructing the spatial distribution of crystalline phases within an intact object requires a diffraction pattern at each point as the sample is translated and rotated through the beam, so a small, intense and well-collimated beam combined with a rapid detector readout is essential to acquire the thousands of patterns involved within a feasible measurement time⁵⁵. High photon energies (short wavelengths) further allow the beam to penetrate thick and dense objects, making measurements of intact objects possible without sectioning.

1.5.1.6. The Cambridge Structural Database

The extensive use of diffraction techniques has generated a large body of structural data, deposited in resources such as the Cambridge Structural Database (CSD). The CSD is the world's largest repository of small-molecule organic and metal-organic crystal structures, curated and maintained by the Cambridge Crystallographic Data Centre (CCDC). Established in 1965, it currently contains over 1.4 million structures⁵⁶. Each entry comes from experimental diffraction data (X-ray, neutron or electron) and contains information on the compound, its crystallographic properties (and physical properties where reported), and the bibliographical source. Entries are organised in a consistent format so that the database can be used not only as an archive but also as a validated and standardised dataset suitable for large-scale analysis. This breadth of high-quality data provides a broader context for interpreting crystal packing and intermolecular interactions⁵⁷.

Because the structures are stored in a common format, information can be easily aggregated across the whole collection. This makes it possible to describe statistically and across thousands of structures the geometric preferences of intermolecular interactions (including directionality), the conformations molecules tend to adopt, and how often specific packing motifs and supramolecular synthons occur⁵⁸. Knowledge-based libraries such as Mogul⁵⁹ (molecular conformations) and IsoStar⁶⁰ (intermolecular interactions) have been created to retrieve this information and assess or predict the likely geometry of systems not yet determined experimentally.

The CSD is interrogated through a suite of CCDC software for fast retrieval, visualisation and analysis of the data. ConQuest⁶¹ enables substructure and geometric searching, while predefined subsets (e.g., the CSD Drug Subset⁶²) narrow a search to

structures of a given class. Mercury⁶³ provides three-dimensional visualisation and structural analysis, including the identification of intermolecular contacts, void space and slip planes. The CSD Python API⁶⁴ (Application Programming Interface) lets the CSD's data and CCDC's analysis tools be accessed and controlled directly from code. The Python API is particularly important for data-driven analysis, as it allows analyses and workflows to be scripted and automated by users, and the database to be mined rather than searched entry by entry⁶⁵.

This combination of data breadth, curation and ability to query it through code has made the CSD well suited to data-analytics and machine-learning approaches in structural science. CSD data are used to train models that relate chemical and structural descriptors to physical properties⁶⁶, and the database is used in crystal structure prediction (CSP); in the most recent CCDC-coordinated international blind test (the 7th CSP Blind Test), machine-learned interatomic potentials were used to rank candidate crystal structures by relative stability⁶⁷. As polymorph stability is a key consideration in solid-form selection⁷, these approaches make the database a basis for predicting new structures and anticipating their properties.

Computational methods such as PIXEL can further support structural interpretation by quantifying intermolecular interactions and their contributions to crystal stability. This approach complements experimental measurements by providing energetic insight into structural changes under compression^{68–70}. PIXEL is an external program that is run directly from the Mercury graphical user interface (GUI) through MrPIXEL, which uses the CSD Python API to set up the calculation⁷¹.

The CSD and its associated tools connect experimentally determined structures to mechanical behaviour. They provide context for interpreting individual structures,

placing a given polymorph or pressure-induced solid form within the broader landscape of known forms and interaction geometries. They also provide structural descriptors such as slip planes, void space and intermolecular contacts that relate a crystal structure to features that inform how it accommodates compression^{63,72,73}.

1.5.1.7. Spectroscopic techniques

Spectroscopic techniques such as Raman⁷⁴ and Terahertz⁷⁵ (THz) spectroscopy provide complementary information by probing vibrational behaviour and intermolecular interactions. These methods are highly sensitive to changes in the local molecular environment and can detect phase transitions and pressure-induced changes in bonding that may not be immediately evident from diffraction data alone^{76,77}. When used alongside diffraction, they provide a more complete picture of structural response under pressure.

When monochromatic laser light interacts with a molecule, a photon briefly raises the molecule to a short-lived virtual energy state before the energy is re-emitted as scattered light (Figure 1.6). Most photons are scattered elastically, with no change in energy (Rayleigh scattering). A small fraction (approximately 1 in 10^6) interacts and exchanges energy with molecular vibrations, and so are scattered inelastically at a shifted frequency. Stokes scattering occurs when a photon of lower energy is scattered, and the molecule is left at a higher vibrational state. Anti-Stokes scattering is the opposite; the molecule begins in an excited vibrational state before interacting with the laser light and is returned to a ground state with the scattering of a photon of higher energy. Vibrational levels are populated following the Boltzmann distribution, so the ground state is more populated at room temperature and Stokes scattering is therefore

more intense than anti-Stokes⁷⁴. Raman spectroscopy measures the inelastic Stokes scattering.

A vibrational mode is Raman active only if it produces a change in the polarisability of the molecule (i.e., its electron cloud deforms). The difference in wavenumber between the incident and scattered light, known as the Raman shift, corresponds to the energy of a particular vibrational mode and provides a vibrational fingerprint characteristic of the molecular structure and local bonding environment⁷⁴. A Raman spectrum plots scattered intensity from each Raman-active mode against its shift.

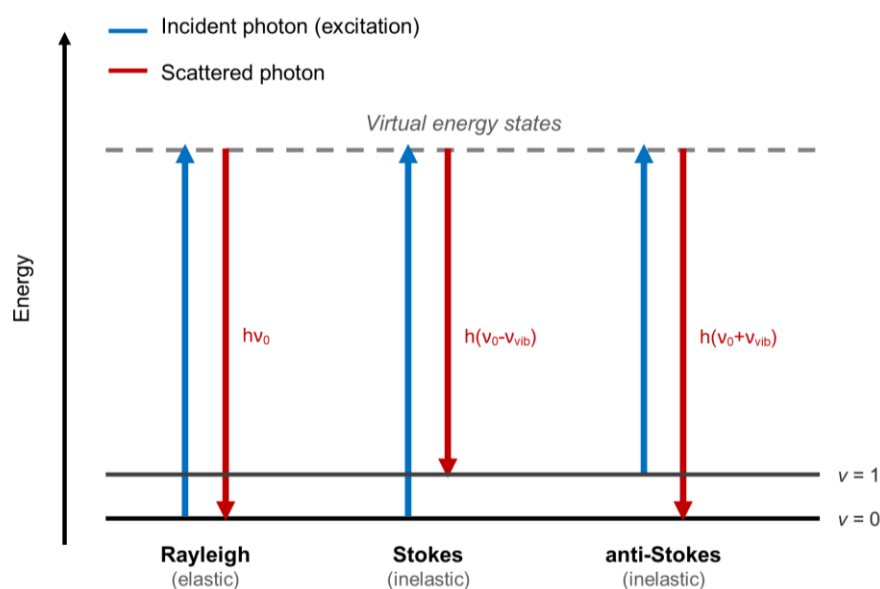


Figure 1.6. Energy level diagram showing Rayleigh, Stokes and anti-Stokes scattering.

When a structure is compressed, its vibrational frequencies generally increase (higher wavenumber) as bonds and intermolecular contacts stiffen within a reduced volume⁷⁶. Changes to the spectra, such as the appearance of a new band, or disappearance or splitting of an existing one, can indicate a pressure-induced change in the crystal structure¹⁴. The lattice modes (vibrations of whole molecules against their neighbours, also called phonon modes) lie in the low wavenumber region (below ca. 200 cm^{-1}) and shift faster under compression than intramolecular modes, making them more sensitive to changes in molecular packing⁷⁶ (Figure 1.7).

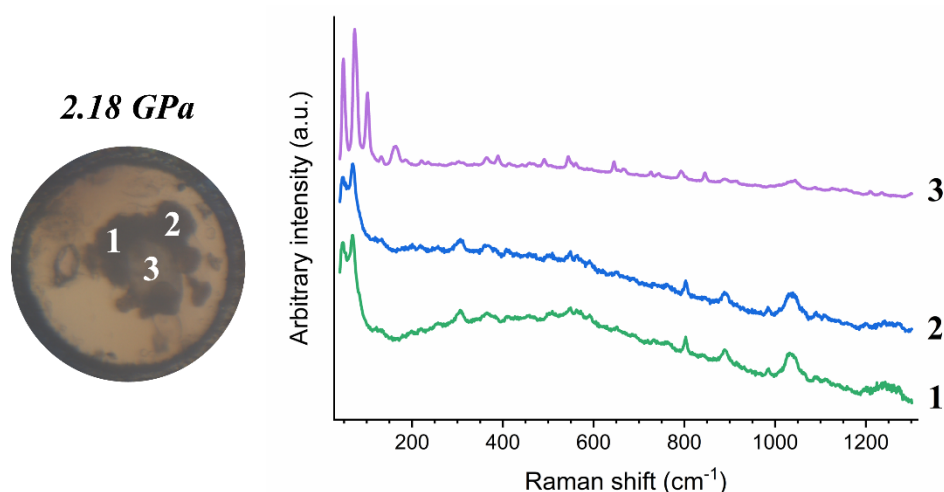


Figure 1.7. Raman spectra of a single crystal of levofloxacin hemihydrate (3) surrounded by a methanol-ethanol mixture at 2.18 GPa and a crystallised new phase (1,2), showing distinct phonon regions below 200 cm^{-1} .

Terahertz spectroscopy is based on the absorption of terahertz radiation, which excites low-frequency vibrational modes. It is particularly sensitive to changes in the collective dynamics of the crystal lattice and intermolecular interactions under pressure. Because Raman and terahertz spectroscopy obey different selection rules (i.e., polarisability for Raman, dipole moment for terahertz/infrared), they probe complementary sets of low-frequency modes⁷⁵.

Additionally, insight into elastic behaviour can be obtained from low-frequency Raman spectroscopy and THz spectroscopy. Both techniques probe collective intermolecular forces and motions within molecular crystals. When combined with X-ray diffraction and Density Functional Theory (DFT) calculations (which describe the electronic structure of a system⁷⁸), Stoll *et al.* used low-frequency Raman spectroscopy to map the intermolecular forces responsible for elastic behaviour across a bent crystal⁷⁹, while Ruggiero *et al.* used THz spectroscopy to calculate the Young's modulus from vibrational force constants⁸⁰.

1.5.1.8. High-pressure studies

Diffraction and spectroscopic measurements are performed on samples compressed at discrete pressure points to follow structural behaviour under pressure. These are referred to as high-pressure studies, reflecting the GPa-range pressures typically accessed.

For high-pressure studies, X-ray diffraction and Raman spectroscopy are commonly performed using a diamond anvil cell (DAC) to monitor structural and vibrational changes *in situ* during compression, respectively⁸¹. A DAC is a device that enables samples to be compressed while enabling access to the sample for X-rays or optical radiation, since diamonds are transparent to both. It comprises two opposing diamonds mounted on backing seats with their culets facing one another (Figure 1.8). A culet is the flat tip at the bottom of a diamond. Because pressure is force per unit area, the small culet area allows a modest applied force to generate very high pressures. A metal gasket with a hole drilled in it, which serves as the sample chamber, is placed between the diamond culets to contain the sample under study along with a pressure-transmitting medium that surrounds it and helps maintain hydrostatic conditions

during compression. Hydrostatic conditions refer to pressure being applied uniformly in all directions. A pressure marker (also referred to as a pressure calibrant) is also included to determine the pressure experienced inside the sample chamber. The sample is compressed between the diamonds and measurements are taken at different pressure points³⁶.

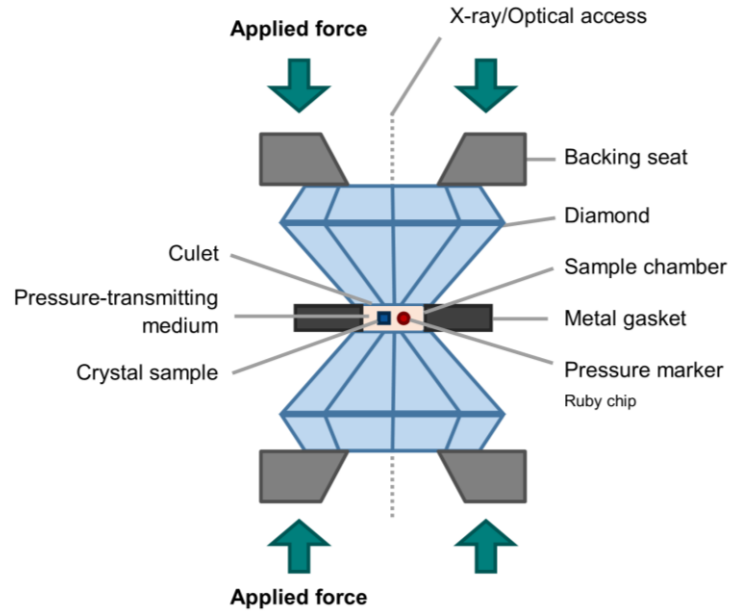


Figure 1.8. Labelled schematic of a diamond anvil cell.

Ruby is a commonly used pressure marker⁸², although others are available such as gold⁸³, which is well-suited for pressure measurements at high temperatures, and NaCl⁸⁴. Using ruby, pressure is determined from the shift in the wavelength of its R1 fluorescence line relative to its ambient-pressure position (Figure 1.9), following the Ruby2020 gauge⁸⁵

$$P = 1.87(\pm 0.01) \times 10^3 \left(\frac{\Delta\lambda}{\lambda_0} \right) \left[1 + 5.63(\pm 0.03) \left(\frac{\Delta\lambda}{\lambda_0} \right) \right] \quad (6)$$

where P is pressure measured in GPa, λ_0 is the wavelength of the R1-line at ambient pressure (≈ 694.25 nm), and $\Delta\lambda$ is its shift under pressure. The relationship between pressure and wavelength shift is approximately linear below *ca.* 20 GPa (i.e., within the pressure range in which pharmaceutical organic materials are typically studied).

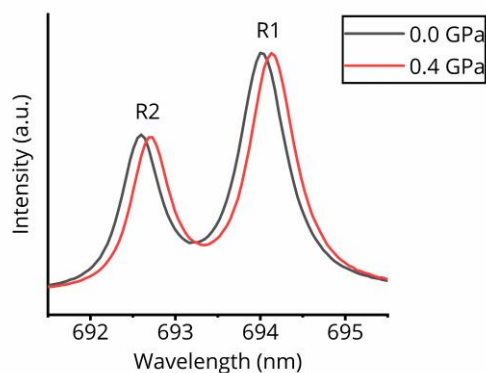


Figure 1.9. Pressure-induced shift of the ruby R1 fluorescence line, used to determine the pressure experienced by the sample in a diamond anvil cell.

Raman spectroscopy is used to flag changes to the crystal structure that may indicate a phase transition. Since measurements are quick, the fast screening of compression studies complements X-ray diffraction. However, a change in the Raman spectrum is not unambiguous evidence of a phase transition; spectral changes can be subtle and open to more than one interpretation, and confirming a transformation generally requires a crystal structure resolved by diffraction, as seen for L-alanine and DL-alanine^{86,87}. Care must also be taken around the 1300-1400 cm^{-1} as the diamonds in the DAC generate a sharp band at *ca.* 1335 cm^{-1} .

The structural response of an API obtained through X-ray diffraction can be quantified through parameters such as bulk modulus, a measure of a crystal's resistance to compression⁸⁸, and the principal axes of strain, which capture the anisotropy (i.e., directional dependence) of that compression⁸⁹.

Organic and metal-organic materials are considered soft materials and have a low bulk modulus within the *ca.* 5 to 50 GPa range (e.g., 10.7 GPa for paracetamol Form I, calculated from ⁹⁰; 25.2 GPa for UiO-66(Zr)-NH₂⁹¹), whereas inorganic materials are much more resistant to compression and therefore considered hard materials; they can reach extremely high bulk moduli of up to *ca.* 440 GPa (e.g., 440 GPa for diamond⁹²).

The bulk modulus is obtained by fitting the pressure-volume data to an equation of state (EoS), such as the Vinet EoS⁹³

$$P = 3K_0 \left(\frac{V}{V_0}\right)^{-\frac{2}{3}} \left[1 - \left(\frac{V}{V_0}\right)^{\frac{1}{3}}\right] \exp\left\{\frac{3}{2}(K'_0 - 1) \left[1 - \left(\frac{V}{V_0}\right)^{\frac{1}{3}}\right]\right\} \quad (7)$$

where P is pressure, V_0 is the material's volume at ambient conditions, V is the volume at pressure, K_0 is the bulk modulus, and K'_0 is the first pressure derivative of the bulk modulus, describing how the material stiffens under compression, defined as

$$K'_0 = \left(\frac{\partial K}{\partial P}\right)_{P=0} \quad (8)$$

The Vinet equation of state can be fitted in a second-order form (where $K'_0 = 4$ is fixed) or in a third-order form (where K'_0 is treated as a free parameter). The choice depends on which form best represents the compression behaviour of the material.

Different forms of equations of state exist, each based on different assumptions about the compression behaviour of the material⁹⁴. Careful consideration goes into choosing the appropriate equation of state; the choice depends on the type of material and the compression range studied, as well as the goodness of fit. Computer programs such as EoSFit facilitate the calculation of an equation of state and the visualisation of its fit to experimental data⁹⁵.

Together, these measurements provide a quantitative account of the relationship between structure and deformation. The principal axes of strain are the orthogonal directions of maximum, intermediate and minimum compressibility in the system, and can be obtained using programs such as PASCAL⁸⁹. While the bulk modulus describes the overall volume response, the principal axes capture the directional compression that dominates in low-symmetry organic crystals. However, determining these parameters is more challenging for organic materials of low symmetry than for high-symmetry inorganic ones. Lower symmetry crystal structures can deform more freely; their unit cell parameters can vary independently under pressure (e.g., all three axis lengths and angles in a triclinic cell, in contrast to a single axis length in a cubic cell), so the structure's deformation is described by more variables. In low-symmetry systems, the principal axes of strain are not fixed to the unit cell axes and can reorient with pressure; therefore, their magnitudes and orientations must be determined in addition to tracking changes in the unit cell⁸⁹. Organic materials are also softer and compress to a greater extent over a given pressure range, so closely spaced pressure points are needed to resolve the curvature of the P - V data and fit the equation of state well⁹⁴. The compression of a low-symmetry structure is also highly anisotropic and strongly dependent on the orientation of applied stress, making it especially sensitive to the pressure environment and changes to it⁹⁶. As such, values measured under hydrostatic conditions do not translate directly to the anisotropic environment of tabletting.

1.5.1.9. Complementarity of techniques

While both diffraction and spectroscopic techniques probe changes in the crystal structure, the results obtained do not always agree. In alanine, early Raman studies reported pressure-induced phase transitions in L-alanine at approximately 2 and 9 GPa. However, subsequent work by Tumanov *et al.* combining X-ray diffraction and Raman spectroscopy showed no evidence of structural phase transitions over this pressure range. The observed changes in the spectra were instead attributed to continuous structural distortions and, in some cases, the formation of solvates⁸⁶. Similarly, Raman studies on DL-alanine suggested phase transitions at 1.7–2.3 GPa and 6.0–7.3 GPa. These were later re-evaluated by Tumanov *et al.* combining X-ray diffraction and Raman measurements on the same sample, which demonstrated that no structural phase transitions occur. The apparent discontinuities in the Raman spectra were explained by bands overlapping and the different pressure dependences of vibrational modes⁸⁷.

The phase transition in naphthalene between 2–4 GPa has also been heavily disputed. Evidence of a transition has been reported by Bridgman⁹⁷ (1938) as a volumetric change in a study up to 3.0 GPa, Block *et al.*⁹⁸ (1970) as an optically visible change within a DAC between 2–3.5 GPa, Hamann *et al.*⁹⁹ (1978) as a discontinuous shift in the IR spectra between 2–4 GPa, Meletov *et al.*¹⁰⁰ (2013) as discontinuous changes in the Raman spectra near 3.5 GPa, and most recently O'Bannon and Williams⁷⁶ (2016) confirmed the subtle transition at 2–3 GPa using Raman and IR spectroscopy. However, a volumetric study by Vaidya and Kennedy¹⁰¹ (1971) found no transition up to 4 GPa. A Raman study by Nicol *et al.*¹⁰² (1975) also found no evidence of a transition up to 3.6 GPa, and Fabbiani *et al.*'s¹⁰³ (2006) single crystal X-

ray study up to 2.1 GPa and Likhacheva *et al.*'s¹⁰⁴ (2014) synchrotron X-ray powder diffraction study up to 5.6 GPa agreed, observing no transition. In 2022, Wilson *et al.*¹⁰⁵ revisited Likhacheva *et al.*'s data and partitioned the unit cell into network volume and void volume. They demonstrated that a subtle transition occurs at ca. 1.9 GPa, marked by a change in the compressibility of the network volume that conventional diffraction analysis had missed.

Overall, diffraction and spectroscopic techniques provide a detailed picture of how a crystal structure evolves under pressure. They describe this evolution through structural descriptors from which molecular rearrangement is inferred: unit-cell parameters and strain tensors from diffraction, vibrational shifts from spectroscopy. A complementary approach that quantifies how molecules translate and reorient relative to one another would add directly to this picture, particularly when relating structural change to mechanical behaviour.

1.5.2. At a macroscopic level

Characterising pressure-induced change at the macroscopic level brings together three themes: the structural features that connect an API's molecular structure to its mechanical behaviour, the measurement of its mechanical behaviour, and the probing of molecular-level structural change within a tablet, where stress is unevenly distributed.

1.5.2.1. Slip planes

Crystallographic slip planes are a structural feature that can link molecular structure and mechanical behaviour. Slip planes “*correspond to crystallographic planes in the crystal exhibiting weakest interactions between adjacent planes. They are typically of the highest molecule density and largest d-spacing when compared to other planes in the same crystal*”, as described by Sun and Kiang in 2008¹⁰⁶. Under applied stress, they allow molecular layers to move relative to one another enabling plastic deformation during compaction¹⁰⁷. Materials with active slip systems (i.e., a slip plane and slip direction) tend to exhibit enhanced plasticity, thereby increasing the interparticulate bonding area and improving the tensile strength and tableability of the resulting tablets^{108,109}. Whether a slip system is active depends on the crystal structure and anisotropy¹¹⁰. Molecular crystals often combine low symmetry with a mix of weaker and stronger intermolecular interactions, so slip tends to favour specific crystallographic directions. As a result, deformation mechanisms can be highly anisotropic^{110,111}. Identifying potential slip planes from a crystal structure is an informative first step and pairing it with experimental and computational analysis establishes which planes are active under realistic conditions (Figure 1.10). This has been demonstrated for molecular crystals including aspirin (forms I and II), where the

geometry-predicted (100) slip plane¹¹² is confirmed to be active by both DFT shear slipping calculations¹¹³ and nanoindentation¹¹⁴, and succinic acid, whose (020) and (111) slip planes predicted by attachment energy calculation were directly confirmed by atomic force microscopy (AFM) nanoindentation¹¹⁵.

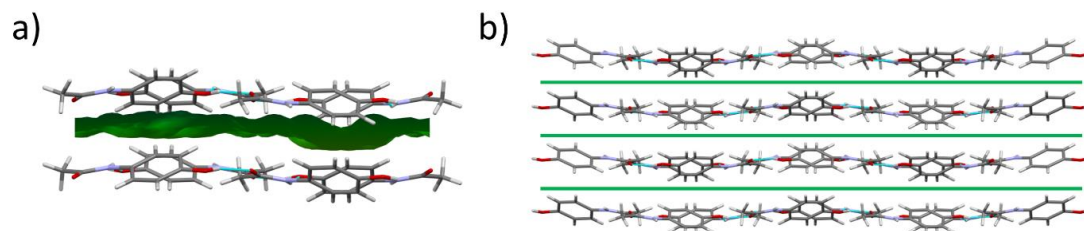


Figure 1.10. The (002) slip plane in paracetamol Form II. The orthorhombic form II contains well-defined slip planes that confer greater plasticity and tabletability to the structure in comparison to Form I^{116,117}. (a) Calculated slip plane geometry within a single unit cell using the CSD Slip Planes tool; (b) expanded packing showing the position of successive (002) slip planes, marked by straight lines.

1.5.2.2. Nanoindentation and Atomic Force Microscopy

Crystal bending and nanoindentation enable mechanical behaviour to be investigated at the particle level^{118,119}. Nanoindentation is widely used to measure the elastic modulus, hardness and deformation behaviour across different crystal faces or polymorphs^{120,121}. When combined with atomic force microscopy (AFM), high-resolution imaging before and after indentation provides additional structural context for interpreting deformation. For example, for sucrose crystals, AFM images after indentation show clear indents, a *ca.* 20-25 nm material pile-up around them, and absence of cracking, consistent with plastic flow through a dislocation mechanism¹¹⁹. To build a comprehensive picture of the mechanical behaviour of an API particle, measurements across several crystal orientations are required, since the response depends on the orientation of the applied stress relative to the crystal^{119,120}.

1.5.2.3. X-ray and X-ray diffraction computed tomography

Moving from particle to tablet, several studies have characterised phase transformations in tablets after compaction using X-ray diffraction¹²². These studies have required destructive sample preparation, such as breaking up the tablet and/or trituration of the powders prior to analysis, which may alter the material under study. Thakral *et al.* were able to reduce the sample preparation by splitting the tablet open and using two-dimensional X-ray diffraction to map the spatial distribution of polymorphs across the tablet²³. By quantifying the relative fractions of chlorpropamide form C and form A as a function of position, they showed that the conversion from form C to form A was most pronounced at the radial surface of the tablet, with a gradient towards the core. This provided important mechanistic insight, demonstrating that regions near the surface experience higher shear stress compared to the interior. They also noted that incorporating microcrystalline cellulose (MCC, 50:50 wt%) into the tablet modestly reduced the extent of polymorphic transformation. However, despite removing the need for trituration, some degree of sample modification was still required to access the internal regions for analysis, as the tablets needed to be physically split, pointing towards a need for non-destructive, spatially resolved techniques capable of probing intact pharmaceutical tablets.

X-ray computed tomography (XCT) offers a non-destructive approach to characterising the internal structure of intact tablets by reconstructing three-dimensional density maps from a series of projections. This enables the identification of features such as density gradients, crack formation and voids that arise during compaction and influence tablet quality (e.g., capping and lamination)^{33,123}, though it provides no crystallographic or molecular-level information. X-ray diffraction

computed tomography (XRD-CT) builds on this approach by adding diffraction-based structural information, so that the crystalline forms within an intact object can be identified and their distribution mapped throughout its volume^{55,124}. This means that the crystalline components can be distinguished and their spatial distribution mapped within the object. Since the measurements are non-destructive, objects can be studied as they are without needing to be prepared or altered beforehand, and they can be remeasured multiple times.

These features make XRD-CT a promising technique for studying compaction in pharmaceutical tablets. Tablets can be analysed as produced, so that structural changes observed relate directly to the tableting process itself rather than to subsequent handling. The spatial resolution allows the effects of stress and strain on the powder bed to be examined directly and compared across compaction conditions, providing a better understanding of how pressure gradients evolve during compaction. Mapping API polymorphic transformations across different formulations and pressures provides information to mitigate unwanted phase changes during tableting. Additionally, the non-destructive nature allows the same tablet to be followed over time, so that processes such as degradation or anhydrate/hydrate formation can be resolved as a function of storage conditions, capturing valuable information for stability testing and shelf-life prediction¹²⁵.

Each of these approaches connects some aspect of molecular structure to mechanical or tablet-scale behaviour. What remains is to bring together molecular-level structural change and behaviour under tableting conditions. The next section sets out the specific differences that make connecting the two experimentally demanding.

1.6. Connecting high-pressure studies with tableting

Experimental conditions differ between high-pressure studies and tableting in how pressure is applied, its magnitude, the sample's form and environment, and the spatial heterogeneity of the response itself. This section sets out these mismatches and the gaps they open between molecular-scale characterisations and the tableting environment.

Pressure application. High-pressure crystallographic studies are commonly conducted under near-hydrostatic conditions, in which a pressure-transmitting medium ensures uniform pressure is applied in all directions, although non-hydrostatic and uniaxial compression are also studied¹²⁶. In contrast, tablet compaction involves anisotropic stresses from the uniaxial punch force and the punch and die wall friction, producing a complex and directionally dependent stress environment. These differences influence polymorph stability and the likelihood of transformation¹²⁷, meaning that observations from hydrostatic studies provide valuable insights but do not directly replicate the behaviour observed during tableting.

Pressure magnitude. High-pressure crystallography typically accesses the GPa range, whereas pharmaceutical tableting operates in the MPa range, up to approximately 300 MPa (0.3 GPa). Reaching the lower pressure regime in a DAC presents its own challenges. On the one hand, the small diamond culet size in a standard DAC (e.g., 600 μm radius) makes fine control of low pressures difficult because applying force over a small area produces disproportionately high pressures. On the other hand, the sensitivity of ruby, commonly used as a pressure calibrant, carries a measurement error of ± 0.05 GPa which complicates obtaining accurate

pressure readings in the low-pressure range⁸⁵. Resolving change at pressures below 0.3 GPa requires an experimental set-up tailored to that range.

Sample form. High-pressure crystallography is often performed on single crystals as SC-XRD gives the most reliable high-pressure crystal structure determination¹²⁸, whereas pharmaceutical processing typically mills APIs into fine powders before they are blended with excipients and compacted into tablets. XRPD extends measurements to powders and accounts for the influence of crystallite size on polymorph stability^{129,130}. However, the reduced data quality and completeness when collecting diffraction data with a DAC make it difficult to obtain reliable structural information *via* full-profile refinement (i.e., the Rietveld method)⁴⁷.

Sample environment. In high-pressure crystallographic studies, crystals are examined in isolation, isolating the API's response. During tableting, API crystals are blended with excipients and the mixture of materials can influence both the local stress distribution and the extent of structural transformation during compaction²⁵. This is a factor to bear in mind when translating single-component results to formulated tablets.

Spatial heterogeneity. Finally, structural response during tablet compaction is itself spatially varied, as set out in Section 1.4. Conventional X-ray diffraction provides spatially averaged information, so the localised structural changes driven by pressure and density gradients are not fully captured. Instead, spatially resolved techniques are required to map how the structural response varies across the tablet.

Together, these considerations point to three opportunities to connect molecular-scale study with the heterogeneous environment of tablet compaction:

- i. To characterise structural response across the anisotropic, sub-0.3 GPa conditions of tableting alongside hydrostatic conditions;
- ii. To quantify molecular displacement directly, beyond the structural descriptors of conventional analysis;
- iii. To resolve spatially heterogeneous transformations within intact tablets.

Ultimately, the macroscopic behaviour of APIs is governed by structural changes at the molecular level. Connecting the two scales makes it possible to understand and ultimately predict how these materials behave during pharmaceutical processing. The aims and objectives that follow set out how this thesis approaches that goal.

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Chapter 2 - Aims and Objectives

2.1. Aims

The principal aim of this work is to advance the understanding of the structural response of pharmaceutical solids to applied pressure, from the molecular to the macroscopic scale, with relevance to the pharmaceutical tableting process.

Pharmaceutically relevant materials are subjected to elevated pressures during processing, up to 0.3 GPa or 300 MPa during tableting, which can alter their solid form and properties. Understanding what governs this response requires characterising structural behaviour across different pressure conditions and magnitudes. Chapter 3 investigates this by using the racemic and chiral solid forms of ofloxacin and levofloxacin as model systems, examining their structural response across hydrostatic and non-hydrostatic conditions and across a pressure range spanning high-pressure to tableting-relevant pressures. It aims to establish how molecular packing and intermolecular interactions govern compressibility, and to identify any pressure-induced phase transitions and the conditions that trigger them. The structural changes resolved by these conventional analyses are constrained by crystallographic symmetry and may overlook subtle molecular-scale rearrangements. This limitation is addressed by MolDis, the method developed in Chapter 4.

Current methods for analysing the structural response under pressure, such as principal axes of strain, are based on unit cell changes and are constrained by crystallographic symmetry, capturing only indirectly how molecules move relative to one another. Chapter 4 addresses this by developing a methodology, implemented as a Python-based tool (MolDis), that calculates and visualises pressure-induced

molecular displacements relative to a reference molecule directly from variable-pressure X-ray diffraction data. The method can be coupled with interaction energy, void and slip plane analyses to provide spatial information on the compression, providing a deeper understanding of structural response beyond what conventional methods offer.

During my PhD I had the opportunity to explore the impact of compression on pharmaceutical materials using a state-of-the-art technique of X-ray Diffraction Computed Tomography (XRD-CT) (Chapter 5). Establishing the solid-state behaviour of an active pharmaceutical ingredient in a tablet is challenging and has conventionally required destructive sample preparation where the tablet is broken up for analysis. This process risks altering the solid form under study thereby potentially providing inaccurate data on the impact of pressure. XRD-CT can examine intact tablets to elucidate the impact of compaction across and above the tableting pressure range. For this study, glycolide was used as a pressure-sensitive marker as it is known to undergo a phase transformation at high pressure. This is the first time this technique has been used for pharmaceutical tablets. Chapter 5 aims to establish whether pharmaceutical materials transform under compaction and whether XRD-CT is a good method to explore these typically low-symmetry organic systems. Both spatial and time-dependent studies were performed, highlighting the importance for explorations of this type.

2.2. Objectives

To achieve the stated aims of this work, the following objectives were investigated:

Chapter 3 – Pressure-Induced Phase Behaviour and Compressibility of the Racemic and Chiral Solid Forms, Ofloxacin and Levofloxacin

- i. To follow the structural response of ofloxacin, γ -levofloxacin and levofloxacin hemihydrate across a range of pressures and compression conditions using X-ray diffraction, with Raman spectroscopy used to confirm solid-form changes where appropriate.
- ii. Identify and compare pressure-induced phase transitions across the systems studied, establishing the pressures at which they occur and the conditions that trigger or suppress them (hydrostatic or non-hydrostatic).
- iii. Rationalise the compressibility and structural response in terms of molecular packing, intermolecular interactions and lattice energies, comparing related solid forms.

Chapter 4 – Analysis of pressure-induced molecular displacement in pharmaceutical crystals

- i. Develop and implement a Python-based tool (MolDis) to calculate and visualise pressure-induced molecular displacement relative to a reference molecule from variable-pressure X-ray diffraction data.
- ii. Validate the methodology against systems with known compression behaviour (paracetamol Form I and Form II).
- iii. Apply and evaluate the methodology on structurally related acetanilide compounds whose high-pressure behaviour is unreported (monoclinic form

of 4-methylacetanilide, 4-chloroacetanilide, metacetamol Form I), relating molecular displacements to intermolecular interactions, void space and potential slip planes.

Chapter 5 – Spatial and Temporal Visualisation of Polymorphic Transformations in Pharmaceutical Tablets.

- i. Apply X-ray Diffraction Computed Tomography to locate pressure-induced polymorphic transformation in intact pharmaceutical tablets, using glycolide as a pressure-sensitive marker.
- ii. Identify and spatially resolve structural heterogeneity that develops across tablets as a function of increasing compaction pressure.
- iii. Evaluate time-dependent structural changes in the tablets during storage, including any chemical or solid-form transformation.

Chapter 3 - Pressure-induced phase behaviour and compressibility of the racemic and chiral solid forms, ofloxacin and levofloxacin

J. Gasol-Cardona, M. R. Ward, D. Comboni, M. Hanfland, R. Scatena, M. R. Warren, A. G. P. Maloney and I. D. H. Oswald, *CrystEngComm*, 2025, **27**, 7662–7676.

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3.1. Abstract

This Chapter reports a comparative high-pressure crystallographic study of the RS- and S-forms of 9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid, encompassing ofloxacin (O), γ -levofloxacin ($L\gamma$), and levofloxacin hemihydrate (LH). Single-crystal and X-ray powder diffraction experiments reveal all three compounds are relatively soft and compressible due to dominant dispersive intermolecular interactions via parallel molecular packing of the main bodies. Each form undergoes distinct pressure-induced phase transitions, with $L\gamma$ exhibiting a low transition pressure at 1.14 GPa, while LH displays a unique sensitivity to the water content of the pressure-transmitting medium. Under inert conditions, LH remains stable up to *ca.* 5.1 GPa before a transition to a lower-symmetry polymorph but using certain media it can undergo a phase transition to a new unidentified phase. O shows subtle structural changes above 4.65 GPa in methanol-ethanol medium, though no definitive phase transition was observed in the single-crystal form. These findings provide critical insights into the pressure-dependent behaviour of fluoroquinolone antibiotics, with implications for solid form selection, formulation design, and mechanical stability during pharmaceutical processing.

3.2. Introduction

Polymorphism in drug substances is an area of great importance for the pharmaceutical industry. The differing physicochemical properties exhibited by different polymorphs, such as solubility, stability or hardness^{1,2}, can determine their use in formulations and may impact their processing and the manufacture of their drug product. Small-molecule active pharmaceutical ingredients (APIs) are widely formulated into pharmaceutical tablets for drug delivery. At various stages of the tablet production process, such as milling, micronisation and compaction, the pharmaceutical powder blend is subjected to pressures that can modify the solid form of the drug substance (up to 300 MPa, 0.3 GPa). Numerous studies have documented instances where APIs undergo polymorphic transformations³⁻⁶, partial amorphisation^{5,7,8} and amorphous crystallisation^{5,9} as pure components or within pharmaceutically relevant formulations after being compressed at pressures relevant to tableting^{10,11}. Pressure has also been used to probe materials, for example: to determine the relative stabilities of polymorphic forms recrystallised under ambient conditions¹²; to investigate the thermal stabilities of these forms under compression¹³; to access polymorphs with low lattice energies predicted using crystal structure prediction (CSP)^{14,15}; and explore new routes to polymorphic forms by varying how pressure is applied¹⁶⁻¹⁹. Therefore, it is imperative to investigate materials under pressure and explore these transformations to better understand their underlying triggers and determine the conditions under which a phase transformation is consistently observed. Only when these conditions are determined can a manufacturing process be designed to avoid or intentionally induce pressure-driven phase transformations. One of the ways in which this can be addressed is by the

systematic investigation of related compounds to observe changes in compression as a function of molecular structure. Chiral compounds and their racemic mixtures are a fantastic option as the molecular framework is largely similar, but the packing is different. Previous research has compared the behaviour of single enantiomers and racemic compounds under high pressure in smaller organic molecules, such as amino acids^{20–24}, sodium tartrate monohydrate²⁵ and, more recently, into more pharmaceutically relevant compounds with 2-(2-oxo-1-pyrrolidinyl) butyramide²⁶.

This study focuses on the RS- and S-forms of 9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid, otherwise known as ofloxacin (racemic form, O) and levofloxacin (chirally pure form, L). The subtle difference in structure gives rise to distinct solid-state landscapes for each compound. For O, one anhydrous form is known and no hydrate crystalline forms have been reported^{27,28}. In contrast, L has two known hydrate forms (hemihydrate and monohydrate^{29–31}) and four anhydrous forms (α , β , γ and δ)^{29,30,32,33}. The anhydrous forms are obtained through the dehydration of their hydrated counterparts, as displayed in Figure 3.1. The α -form ($L\alpha$) can also be obtained by recrystallising levofloxacin hemihydrate (LH) in DMSO³³, and the γ -form ($L\gamma$) can also be obtained by neat ballmilling LH at room temperature³⁴. While both hydrates have well-determined crystal structures, the anhydrous forms are unstable at ambient conditions and susceptible to hydration. Consequently, only the crystal structure of $L\alpha$ has been successfully solved by single crystal X-ray diffraction, as well as by structure determination from X-ray powder diffraction data (SD-XRPD) at high temperature^{32,33}. The crystal structure of $L\gamma$ has also been solved by SD-XRPD from

high-temperature data³², but the structural information of the β and δ forms ($L\beta$, $L\delta$) is yet to be obtained experimentally.

One of the critical attributes of O and $L\gamma$ is that there are no intermolecular hydrogen bonds with the key intermolecular interaction being π -stacking. Even the more stable hemihydrate structure utilises this interaction motif with the water bridging between the stacks. This commonality in packing makes this an interesting system to explore under compression where there are subtle changes to the crystal structure and how the solids evolve under the application of pressure. The behaviour of O, $L\gamma$ and LH has been followed through a combination of single-crystal and X-ray powder diffraction to track the compression of the solids and gain a fundamental understanding of the effect of compaction pressure on the API.

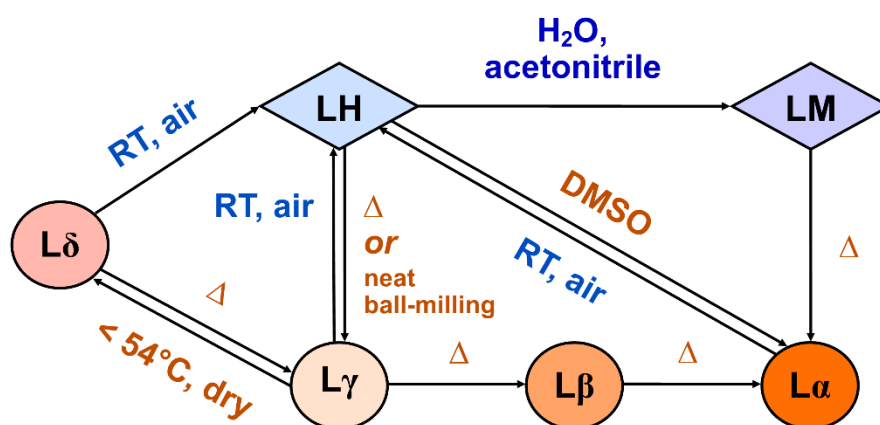


Figure 3.1. Conversion routes between the hydrate (hemihydrate, LH; monohydrate, LM) and anhydrate ($L\alpha$, $L\beta$, $L\gamma$, $L\delta$) crystal forms of levofloxacin. Orange shades: anhydrous forms and conversion conditions. Blue shades: hydrate forms and conversion conditions.

3.3. Materials and Methods

3.3.1. Materials

O and LH were purchased from Alfa Aesar ($\geq 98.5\%$ and $\geq 97.5\%$ purity, respectively) as polycrystalline powders. The phase and chemical purity of the compounds were examined in-house by X-ray powder diffraction (XRPD) prior to study and recrystallisation (Figure A3.16). The solvents used for recrystallisation were purchased from Alfa Aesar (nitrobenzene) and VWR International Ltd. (acetonitrile).

3.3.2. Powder preparation

O and LH samples were used without further treatment. L γ was prepared by heating LH in a drying oven at 150 °C for 24 h following the approach reported by Freitas *et al.*³². After heating, the sample was immediately sealed in a vial with a screw cap and Parafilm to minimise exposure to atmospheric water to limit the rehydration.

3.3.3. Single crystal preparation

Single crystals of LH were obtained with relative ease whereas the crystallisation of O required the use of a Crystal16 to enable controlled heating and cooling cycles.

40 mg (0.11 mmol) of LH was dissolved in 2 mL of high-performance liquid chromatography (HPLC) grade acetonitrile at room temperature. The solution was left to slowly evaporate and, over the course of 10 days, colourless elongated plate-like crystals appeared in solution.

10 mg (0.03 mmol) of O and 1.2 g (9.76 mmol, 1 mL) of nitrobenzene were weighed into a 1.5 mL vial (VWR-548-0018), and the suspension was heated to 70 °C at 0.3 °C min⁻¹ and held for 30 min with stirring. The suspension was then cooled to 10 °C at 0.3 °C min⁻¹ and held for 30 min. Two further heating–cooling cycles were

performed using the same parameters without stirring. Colourless needle-like crystals were obtained from the solution.

3.3.4. Diamond anvil cell preparation

3.3.4.1 Powder

High-pressure compression studies on powder samples were conducted using membrane-driven diamond anvil cells (mDACs) on beamline ID15B at the European Synchrotron Radiation Facility (ESRF)^{35,36}. The mDACs were equipped with Boehler-Almax anvils and 600 μm culets. For each experiment, a stainless-steel gasket was indented to an 80 μm thickness and a 300 μm diameter hole was drilled through the gasket by spark erosion to serve as the sample chamber.

Each sample was loaded to the sample chamber as purchased or prepared, without further grinding. A ruby sphere was also loaded to serve as the pressure marker, with a pressure uncertainty of ± 0.05 GPa³⁷. The pressure inside the mDAC was calibrated in situ using the ruby-fluorescence method^{38,39}. When exploring isotropic compression, a pressure-transmitting medium (PTM) of either petroleum ether or 4:1 methanol-ethanol was loaded to provide a hydrostatic environment. Both media remain hydrostatic within the pressure ranges explored in this study. The hydrostatic limit of petroleum ether is 6 GPa⁴⁰ and of a 4:1 methanol-ethanol mixture is 10.4 GPa^{41,42}.

3.3.4.2 Single crystal

High-pressure compression studies on single crystals were conducted using Merrill-Bassett DACs equipped with Boehler-Almax anvils with a 40° opening angle and 600 μm culets. Metal foils were used as sample chambers (O, stainless steel indent

130 mm, LH tungsten 120 mm). In each case, a 250 μm diameter hole was drilled using an Almax EasyLab μ Driller. The sample chamber was loaded with a single crystal and a ruby chip. A 4:1 methanol-ethanol mixture was used as the pressure-transmitting medium. Curiously, for compression of LH, repeated experiments using 4:1 methanol-ethanol as the PTM caused the crystal to deteriorate significantly. Five loadings were conducted with a similar result, while crystal quality was maintained in a loading in a 16:3:1 MeOH:EtOH:H₂O mixture indicating that the water content in the PTM may play a key role in stabilising the LH structure. Further explanation is found in Section 3.4.3.2. Further single crystal experiments were conducted using petroleum ether to maximum pressure of 5.8 GPa. The crystallographic data can be found in the Appendix from Table A3.4 to Table A3.11.

3.3.5. In-house X-ray powder diffraction

The samples were lightly ground into a fine powder prior to testing. Data were collected on a small amount of sample (10–50 mg) at room temperature (290–293 K). X-ray powder diffraction data were collected on a Bruker D8 Discover diffractometer configured in a Debye–Scherrer transmission geometry using a Cu sealed tube X-ray source ($K\alpha_{1,2}$ $\lambda = 1.54060$ Å), operated at 40 kV and 10 mA, paired with a focusing Göebel mirror. The diffractometer was equipped with a SSD160-2 1D detector and an automated multiposition x - y sample stage. The samples were placed on a 40 position well plate lined with a 7.5 μm Kapton film and were scanned across a 5–35° 2θ range with a 0.017° 2θ step size and a 4 s per step count time. Throughout the data collection, the samples were oscillated in the x - y plane at a speed of 0.03 mm s⁻¹ to maximise the sample area probed and minimise preferred orientation effects.

The diffraction patterns were analysed in DIFFRAC.EVA⁴³ before fitting the patterns via Pawley refinement in TOPAS V5⁴⁴ using CSD entries with “best” R-factor (i.e., CUYCEF²⁷, LICWOM³², YUJNUM01³⁰) as the initial unit cell parameters. A good agreement (*ca.* $R_{wp} < 4.5$) was observed for ofloxacin and levofloxacin.

3.3.6. Synchrotron X-ray powder diffraction at high pressure

High-pressure X-ray powder diffraction data were collected at room temperature using a 30.0 keV ($\lambda = 0.410691 \text{ \AA}$) synchrotron X-ray source with a horizontal and vertical beam size of $30 \times 30 \mu\text{m}^2$ and a large area DECTRIS Eiger2 X 9M CdTe ($340 \times 370\text{mm}$) flat panel detector placed 300 mm from the sample; the detector parameters were calibrated using a Si standard. The data collection strategy consisted of a single -5° to $+5^\circ$ ω -scan with a 10° step size and a 0.2 s exposure time. After each pressure increase, the sample was allowed to stabilise for 30 min before taking a measurement. The pressure was recorded before and after each pressure step to determine the average pressure experienced throughout the measurement. The pressure limits for each study were dictated by the quality of the diffraction patterns. Diffraction patterns were also collected on decompression.

The 2-D images were visually inspected and integrated using DIOPTAS⁴⁵. Single crystal reflections from the ruby, diamond anvils and spurious peaks were masked before integration. The diffraction patterns were fitted with the Pawley method using the TOPAS software (V5)⁴⁴ to extract the unit cell parameters at each pressure. Unit cell parameters from the previous pressure point were used as starting parameters for subsequent refinements.

3.3.7. In-house single-crystal X-ray diffraction at high pressure

High-pressure single-crystal X-ray diffraction (SC-XRD) data were collected at room temperature on either a Bruker Kappa APEX II diffractometer equipped with a 4-circle kappa goniometer and a CCD detector or D8 Venture coupled with a Photon III detector. Both systems used a Mo Incoatec Microfocus Source ($I\mu S$, $K\alpha_1$, $\lambda = 0.71073 \text{ \AA}$) where the latter was a $I\mu S$ Diamond source.

The diffraction data were processed using the APEX3 and 5 software package. The data were integrated employing the dynamic masking procedure in SAINT, and absorption corrections were applied using SADABS⁴⁶. The structures were refined with SHELXL⁴⁷ in Olex2-1.5⁴⁸ using a full-matrix least squares on $|F|^2$ refinement. The atomic coordinates from CSD refcode CUYCEF (ofloxacin) or YUJNUM02 (levofloxacin hemihydrate) were used as the reference model. The refinements were conducted using the CX-ASAP pipeline to ensure consistency of approach through the series of models.⁴⁹ The non-hydrogen atoms in O and LH (second loading) were refined anisotropically and isotropically, respectively. RIGU⁵⁰ and DFIX restraints were used where appropriate. The bond lengths were based on those of YUJNUM02³¹ extracted using MOGUL to ensure that the model was chemically reasonable⁵¹. All hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms.

3.3.8. Synchrotron single-crystal X-ray diffraction at moderate pressure

Moderate-pressure compression studies on single crystals were conducted using the sapphire capillary pressure cell⁵² on beamline I19 at the Diamond Light Source (DLS), the UK's national synchrotron science facility⁵³. The sapphire capillary cell enabled the exploration of the pressure regime from 1 bar to 1200 bar. Capillaries 30

mm in length and diameters of 1.4 mm OD $\times$ 1.0 mm ID and 1.0 mm OD $\times$ 0.6 mm ID (ID = inner diameter, OD = outer diameter) were used in this study.

Single crystals were mounted on a strand of Mylar® film (*ca.* 250 – 400 μ m width) using a minimal amount of Loctite® Super Glue to prevent the crystals from moving during the loading of the pressure-transmitting fluid (Figure 3.2). The standard process was modified by using narrow pieces of Kapton film rather than the previous method of carbon fibres. The advantage of this technique was that the crystals could be visualised better to enable quicker centring. Small cuts were created in the film so that these could also be used to aid alignment. Two to three crystals were loaded so that data could be collected on multiple crystals at every pressure point by moving the z-stage of the goniometer, reducing the time spent loading cells.

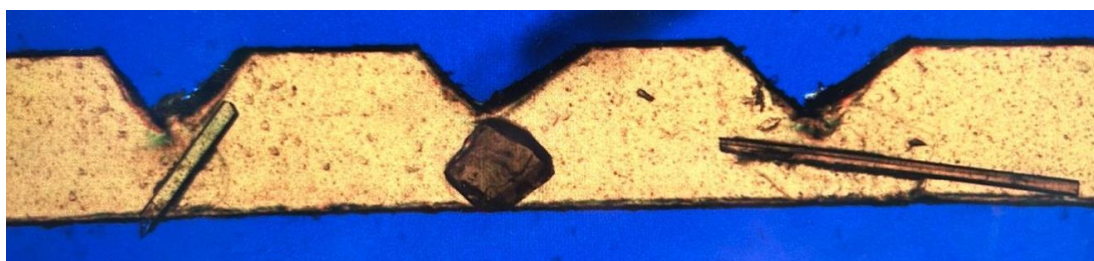


Figure 3.2. Kapton film modification developed during beamtime to reduce the downtime for loading.

The strand was inserted into the capillary and secured making sure the opening of the capillary was not sealed shut. The capillary was filled with Fluorinert™ FC-70 to provide a hydrostatic environment. FC-70 was chosen over the PTMs used in the DAC work due to its lower volatility. Volatility of the medium is particularly challenging with the SCC due to the narrow sample chamber and increased propensity to form air bubbles during assembly.

Moderate-pressure single-crystal X-ray diffraction data were collected at room temperature using a 25.514 keV ($\lambda = 0.48590 \text{ \AA}$) synchrotron X-ray source with a horizontal and vertical beam size of $100 \times 100 \text{ }\mu\text{m}^2$, a Newport 4-circle kappa goniometer and a DECTRIS Eiger2 4M CdTe detector at an 88 mm distance. Crystal centring and data collection were performed using DLS' in-house generic data acquisition (GDA) software. To enhance visualisation for centring it was necessary to fill the capillary with PTM to 20–30 bar depending on loading. Data was collected using a standard collection strategy developed for the sapphire capillary cell, detailed in Table 3.1, the data were reduced using xia2⁵⁴ with DIALS (Diffraction Integration for Advanced Light Sources)⁵⁵, as well as CrysAlisPro, to obtain the best integrated intensities for the samples. This included data integration, absorption correction and space group determination. The structures were refined as in-house collections. Data were collected on samples between 20 bar to 1200 bar (0.12 GPa).

Table 3.1. Data collection strategy used for the sapphire capillary pressure cell⁵².

Scan circle (ϕ)	ϕ
Other axis (ω , $^\circ$)	-79
κ ($^\circ$)	-45
2θ ($^\circ$)	0
Scan ($^\circ$)	-170 to 175
Step size ($^\circ$)	0.2
Exposure time (s)	0.2

3.3.9. In-house Raman spectroscopy

Raman spectra were collected within the diamond anvil cell to confirm the solid-form change observed for levofloxacin hemihydrate (Figure 3.12). This was done using a Horiba XPlora+ Raman microscope with a 785 nm laser. Data were collected across the 40 to 1300 cm^{-1} region using a 1200 gr/mm grating, 100 μm slit and 500 μm hole. The accumulation and acquisition time per collection was adapted to maximise the signal-to-noise ratio. The areas explored in the sample were focused on using a long working distance (LWD) x50 objective. The LabSpec 6 program was used to collect and analyse the spectra.

3.3.10. Structural analysis

Molecular and crystal structure analysis was carried out using the CCDC's Mercury v. 2022.3.0 software⁵⁶. Void space analysis was carried out using the Voids feature in Mercury. The voids were probed using a 0.6 Å probe radius and a grid spacing of *ca.* 0.2 Å. These are non-standard values for this function. With the standard values, the calculated void space rapidly tends to zero hence using smaller values it enables the changes in the void volume to be followed as a function of pressure over the entire pressure range.

The semiempirical PIXEL method, developed by Gavezzotti⁵⁷, was used to explore the intermolecular interactions and lattice energies in the structures studied^{58,59}. PIXEL calculations were performed facilitated by the MrPIXEL program⁶⁰ through the Mercury graphical interface. Hydrogen positions were normalised to conventional neutron values since the structures were determined by X-ray diffraction. Molecular electron densities within a cluster of molecules within a 14 Å radius from a central molecule were calculated using Gaussian09W⁶¹ at the B3LYP/6-31G** level of

theory⁶². A condensation level of 4 was used. For O, Pixel calculations were carried out using the Pixel-C program within the CLP-Pixel package. Standard Pixel calculations were not possible for LH as the structure has a $Z' = 2$. Instead, the Pixel-D method was used, and separate Pixel calculations were carried out between pairs of molecules to obtain individual dimer energies. The symmetry operations relating molecular pairs were visualised in Mercury.

The equation of state (EoS) parameters were obtained using the EoSFit7-GUI software⁶³. The error in pressure was set to 0.05 GPa for high-pressure data³⁷, and 0.00 GPa for moderate-pressure data. The *ca.* 1 bar error associated to pressure readings on the SCC instrument was considered to be negligible. The unit cell volumes of the samples collected in-house under ambient conditions were used as V_0 in the absence of an ambient pressure point in the datasets. The principal axes of strain were calculated using PASCAL^{64,65}, and visualised with the structure in VESTA v3.5.7⁶⁶.

3.4. Results and Discussion

3.4.1. Structural analysis of ofloxacin and levofloxacin

The 9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid molecule consists of an *N*-methylpiperazine ring attached to a rigid, predominantly planar ring system, referred to as the main body. The benzoxazine ring on the main body features a chiral carbon (C12) bonded to a methyl group (C13) (Figure 3.3a). The distinction between enantiomers arises from the chirality at C12 relative to the benzoxazine ring. The O and LH structures display similar reduced cell parameters before unit cell centring operations are applied (Table A3.3). While the molecules in the anhydrate structures take one conformation, LH has two symmetry-independent molecules in the asymmetric unit. These are referred to as the A and B conformers (Figure 3.3b). The key alterations in each of the forms is around the placement of the C13 methyl group on the chiral centre but also in the orientation of the *N*-methylpiperazine ring (Figure 3.3b)^{27,32}.

The *N*-methylpiperazine ring shows significant differences between the phases, with the mean planes between the piperazine (N2–C14–C15–N3–C16–C17–C18) and main backbone (C4–C5–C6–C7–C8–C9) varying from 54.7° to 71.4° for LH (A conformer) and L γ , respectively. The distribution of this torsion angle in the CSD is broad (e.g., min 37.6, max 77.5°) with the mean of 54(9)° ($n = 96$) indicating that the conformation of LH is representative of this distribution and that L γ is observed in the upper quartile. In all three structures, the molecules stack using the $\pi \cdots \pi$ interaction afforded by the main body of the molecule. Although the degree of overlap varies, these interactions make a significant contribution to the overall energy of the system

and are comparable with hydrogen-bonding interactions (Figure 3.3c–e and Table 3.2)^{67,68}. Interestingly the intermolecular energy of the stacked dimer in *L* γ is almost half that of LH indicating the sub-optimal packing of the molecules that contributes to its instability ($-48.8 \text{ kJ mol}^{-1}$, *cf.* -90.1 or $-91.3 \text{ kJ mol}^{-1}$).

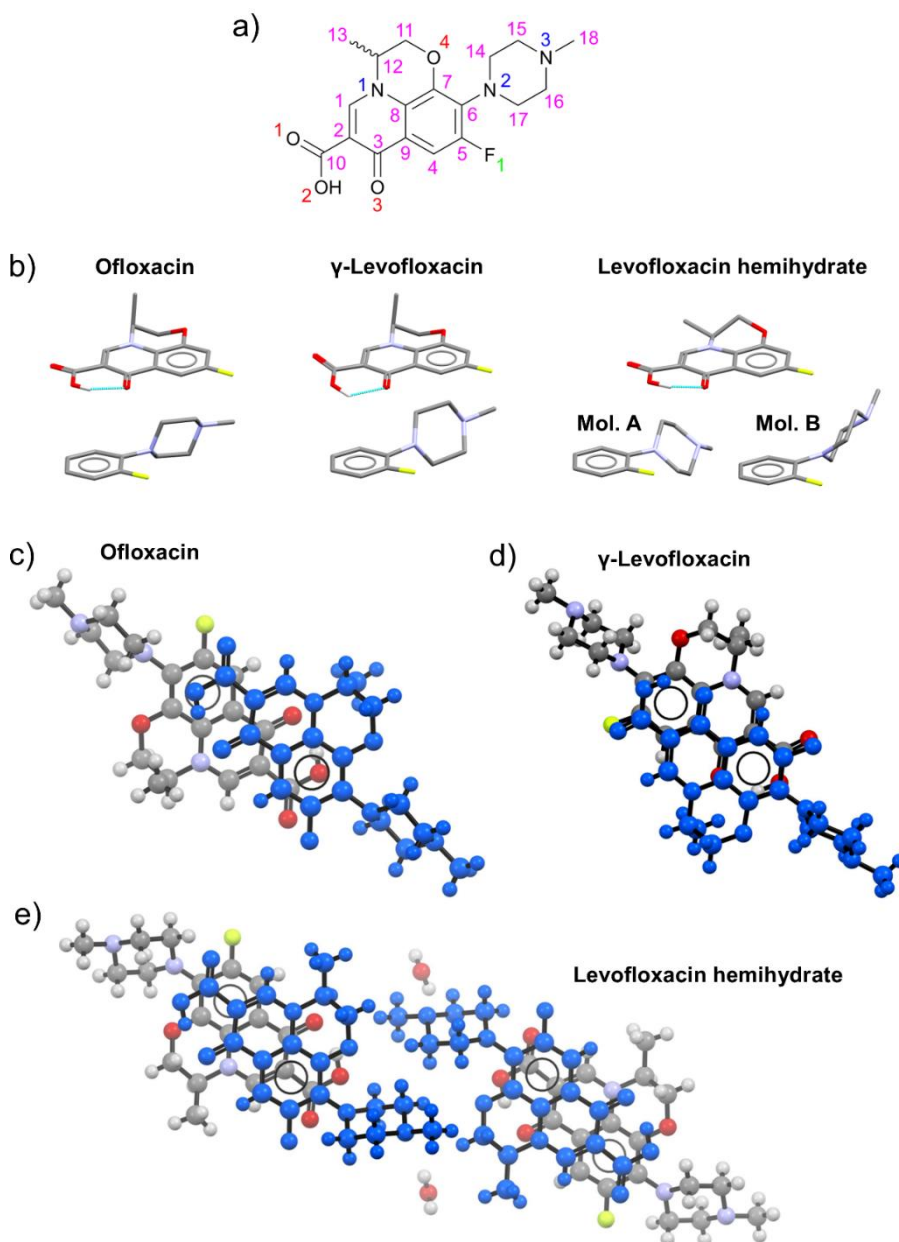


Figure 3.3. (a) Molecular structure of 9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid and the atomic numbering scheme used. (b) Conformation of the piperazine groups in *O*, *L* γ and LH. LH has two molecules in the asymmetric unit. The first molecule of LH (Mol. A) has piperazine above plane and the second molecule (Mol. B) pointing into the plane. (c–e) π -Stacking observed in *O*, *L* γ and LH showing the overlap of the main body of the molecule. This is one of the most significant interactions across all crystal structures.

Table 3.2. Total interaction energy (E_{tot}) and distance between centres of aromatic rings ($\text{Cg}\cdots\text{Cg}$) for the interaction between two stacked molecules along the b -axis in the systems calculated using Pixel^{58,69}. Due to inversion symmetry, the two molecules of ofloxacin can stack with the C13 methyl groups pointing outwards or towards each other (inwards). Total lattice energies for O are found in Table A3.12 and at pressure Table A3.15.

	Symmetry code	E_{tot} (kJ mol ⁻¹)	Aromatic Cg $\cdots$ Cg distance (Å)
O (outwards)	$-x, -y, -z$	-76	4.011
O (inwards)	$-x, 1-y, -z$	-71.2	4.664
L γ	$\frac{1}{2}-x, \frac{1}{2}+y, -z$	-48.8	4.382
LH(A $\cdots$ A)	$\frac{1}{2}-x, \frac{1}{2}+y, -z$	-90.1	3.904
LH(B $\cdots$ B)	$\frac{1}{2}-x, \frac{1}{2}+y, 1-z$	-91.3	3.977

Overall, the three crystal structures studied exhibit great similarities in packing. The molecules are related by a 2₁-screw axis and stack head-to-tail in columns along the b -axis^{27,32}. In O, neighbouring columns along the c -axis form a herringbone pattern with weak C $\cdots$ O interactions with a R₂²(14) graph-set notation (Figure 3.4A)^{27,32}. In this case, neighbouring molecules are not planar with respect to each other but lie at an angle of 23° to each other. In the levofloxacin cases neighbouring groups lie parallel to one another albeit that in L γ they are offset along the c -direction. It is striking that the layers across all three structures are very similar only deviating through subtle translations along the c -axis. There are CH $\cdots$ F contacts present linking stacks at a distances longer than those observed in other substituted systems^{14,70,71}. The lattice energies indicate that O is more stable than L γ (Table A3.12; Figure A3.17; -156 kJ mol⁻¹ vs. -112 kJ mol⁻¹) through higher dispersive contributions of the stacking interactions which contribute to the overall energy.

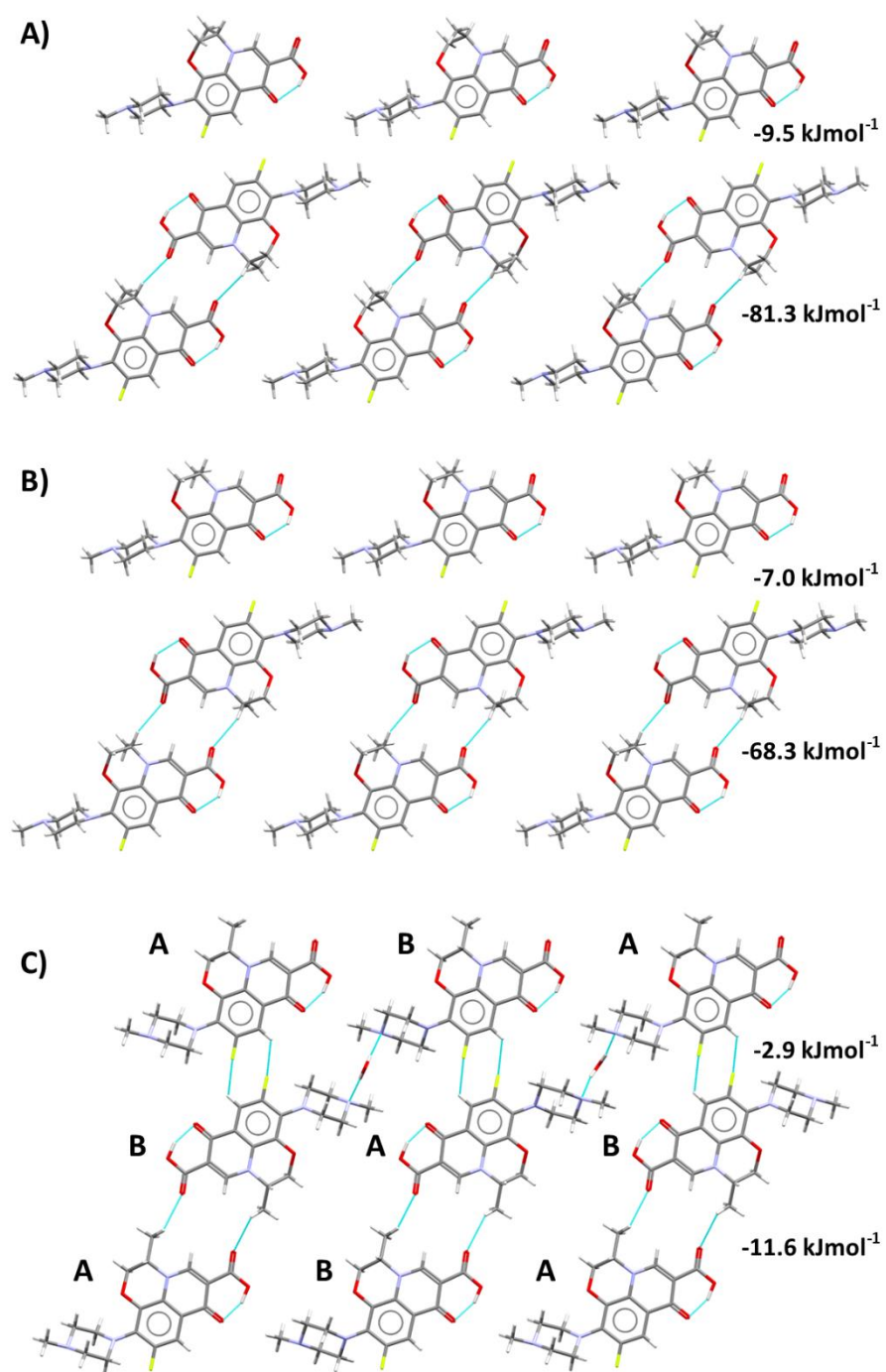


Figure 3.4. The intermolecular contacts between neighbouring molecules in the ac plane are indicated by cyan bonds in (A) O, (B) L γ and (C) LH. The energies between the molecular pairs, as calculated using PIXEL, are provided.

3.4.2. Hydrostatic compression

The structural behaviour of O, L γ and LH was explored via powder and single crystal X-ray diffraction. The use of diamond anvil cells and the sapphire capillary cell provided a comprehensive overview of the behaviour over a wider range of pressures. For the DAC investigations, two pressure-transmitting media (PTM) were used to follow the structural changes: petroleum ether and 4:1 methanol-ethanol. The materials investigated are insoluble in petroleum ether and only slightly soluble in 4:1 methanol-ethanol^{72–75}. The latter medium was chosen because the solubility enhancement can facilitate access to high-pressure solid forms by using solvation energy to overcome the kinetic barrier to conversion⁷⁶. The sapphire capillary cell experiment was limited to the use of FC-70 due to the volatility of petroleum ether and the solubility in methanol-ethanol.

All three fluoroquinolones are highly compressible and soft materials. Their bulk moduli, calculated from methanol-ethanol data (Figure 3.5, Table A3.13), fall at the lower end of the reported compressibility range for organic materials (O 8.0(6), L γ 12.6(14), LH 5.6(8) GPa) [2nd order Vinet over range 0–0.8 GPa], which are typical for a van der Waals solid, for example ROY OP has a bulk modulus of 4.3(3) GPa⁷⁷. Other pharmaceutically relevant materials show a varied degree of stiffness (*R,S*-ibuprofen 4.9(7) GPa, calculated from ref. ⁷⁸; chlorothiazide Form I 23(2) GPa, calculated from ref. ⁷⁹ (2nd order); and paracetamol Form I 10.7(6) GPa, calculated from ref. ⁸⁰). Those higher values related to the hydrogen-bonding present in the material.

The powder data, and the fits to the unit cell volumes, indicate that O and LH compress without any obvious change to the crystal structure up to the maximum

pressure (Figure 3.6). The Pawley fitting of the data of $L\gamma$ indicates that the structure undergoes a subtle phase transition between 0.87 and 1.14 GPa due to the presence of a discontinuity in the unit cell parameters. The unit cell parameters at ambient conditions are taken from the in-house study; therefore, the discontinuity between ambient pressure and 0.31 GPa cannot be ruled out as originating from differences in experimental set-ups. Although the compression of O and LH could be followed using single crystal diffraction, single crystals of $L\gamma$ could not be obtained, nor could Rietveld analysis be performed due to lack of powder averaging due to the small sample size, restrictions of the DAC and subsequent data collections (Figure A3.21). Nevertheless, Pawley refinement has enabled tracking of changes in the unit cell parameters in $L\gamma$ and calculation of the principal axis of strain to assess the impact of pressure on the crystal structure.

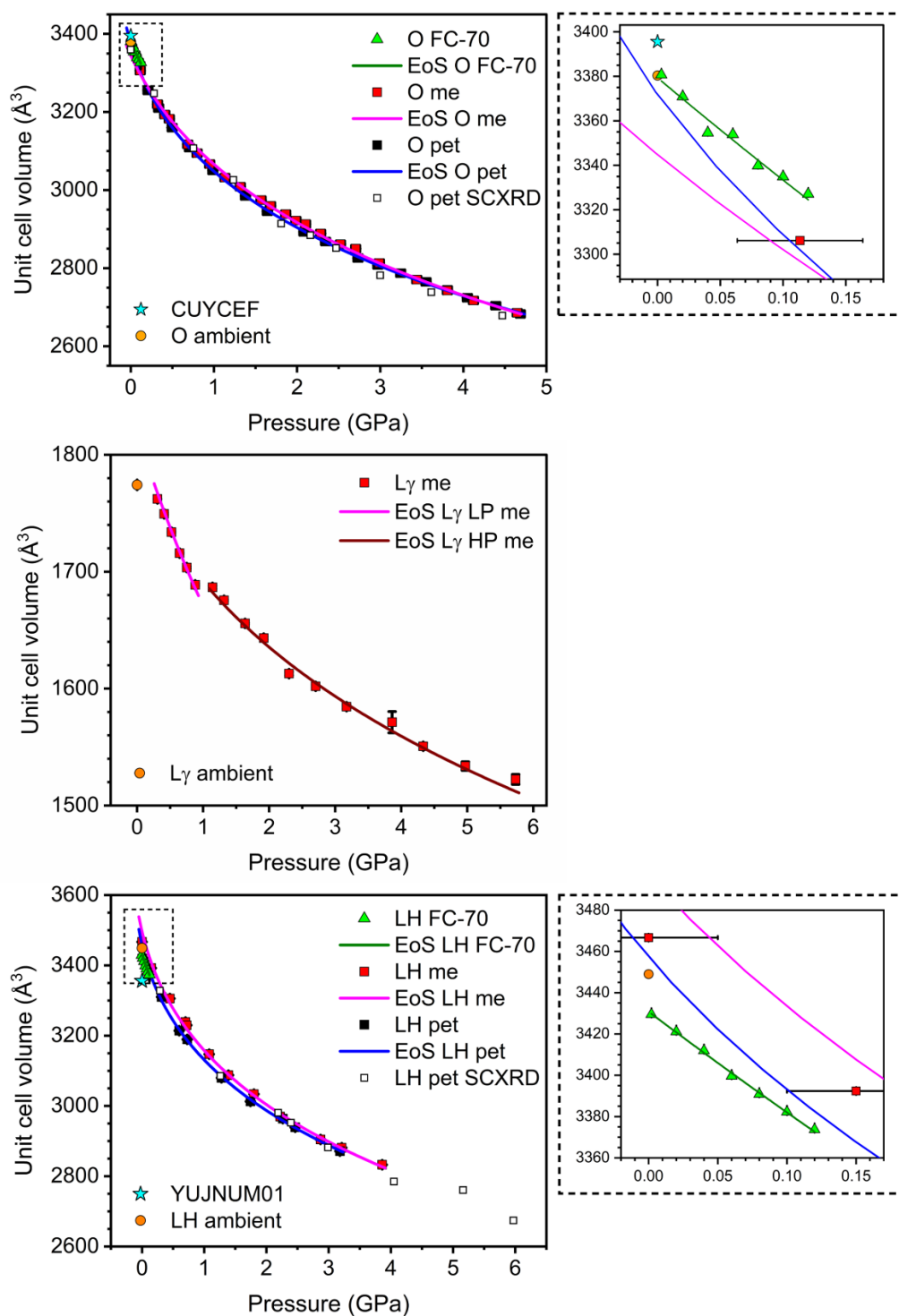


Figure 3.5. Change in the unit cell volume of ofloxacin (O), γ -levofloxacin ($L\gamma$) and levofloxacin hemihydrate (LH) as a function of pressure. Data points include errors in unit cell volume and pressure. Volumes were fitted using the equations of state listed in Table A3.13. For $L\gamma$, volumes before and after the suspected phase transition were fitted separately as low-pressure (LP) and high-pressure (HP) phases. Unit cell volumes of CSD entries for ofloxacin (CUYCEF) and levofloxacin hemihydrate (YUJNUM01) are included. Unit cell volumes of ambient in-house XRPD data are included. Samples were compressed in Fluorinert TM FC-70 (FC-70), a methanol-ethanol mixture (me) and petroleum ether (pet). Overlaid are the unit cell volumes for the single crystal measurements (black unfilled squares). For LH, the unit cell volume of the high-pressure phase is multiplied by 2 to equate to the low-pressure form.

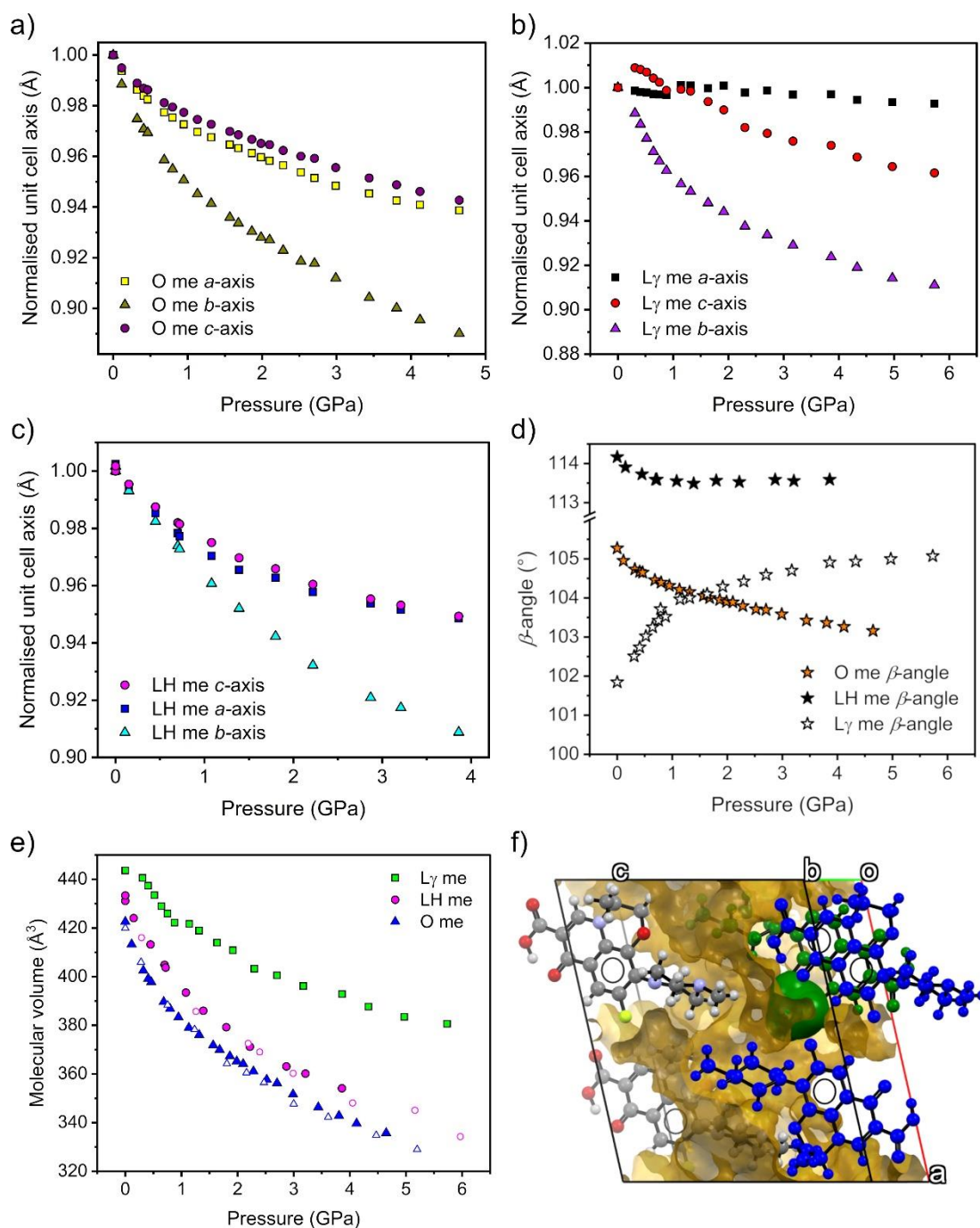


Figure 3.6. a–e) Change in the unit cell axes, β -angle and molecular volume of O, L γ and LH as a function of pressure. The samples were compressed in a 4:1 methanol-ethanol (me) medium. The molecular volume was calculated by dividing the unit cell volume by the number of molecules in the unit cell, Z (i.e., 4 for O and L γ , 8 for LH). The molecular volumes of the samples at ambient conditions are determined from the in-house measurements. Hollow symbols are data from SC-XRD. f) A molecular packing diagram of γ -levofloxacin with the void space (0.6 Å probe radius and a grid spacing of 0.2 Å) indicating the space where the methyl group (green) is likely compressed into during compression.

On compression of $L\gamma$ the b -axis, which is perpendicular to the mean plane of the molecule, is the most compressible direction which is a common observation amongst the compounds studied. At 1.14 GPa, $L\gamma$ shows a discontinuity in the unit cell volume that can be traced to the elongation of the a - and c -axes. This behaviour can be rationalised using the ambient structure as a guide. In the ambient structure, the methyl group (C13) on the chiral carbon (C12) protrudes in-between molecules in the layer below (green space-filled atoms in Figure 3.6f). The compression of the unit cell, and particularly the b -axis, would force the methyl group (green molecule) into the void space between the molecules in the layer below (blue molecules). This movement would require the blue molecules to separate, therefore extending the structure along the a - and c -axes.

The median principal axis of strain lies on the ac plane and is oriented diagonally to the a - and c -axes (Figure 3.7a; Table A3.14). This can be correlated to the changes in the β -angle of the unit cell on compression. As the structure is compressed the molecules at $a = \frac{3}{4}$ in the figure move towards those at $a = \frac{1}{4}$ which has the effect of a shearing interaction between row 1 and 2 in effect increasing the β -angle. This movement is facilitated by the large void volume present between the molecules (Figure 3.7c). The compression along the a -axis is restricted because the molecules on the same ac plane are parallel to each other causing edge-to-edge interactions. This has been observed in other planar molecules⁸¹.

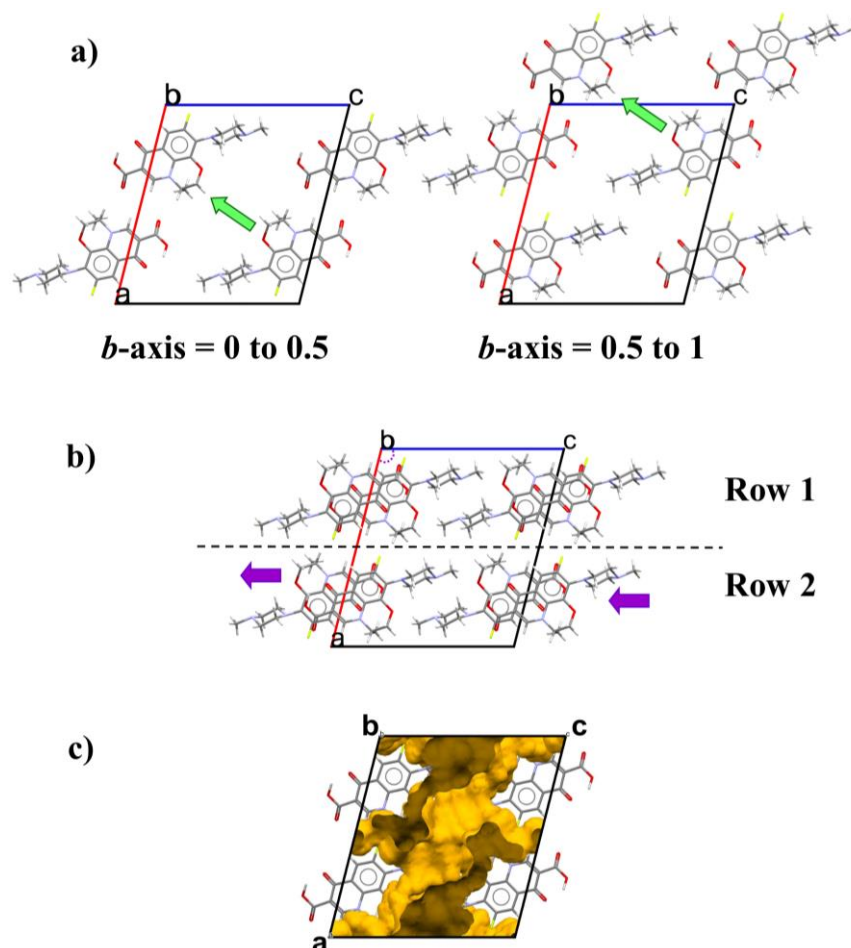


Figure 3.7. (a) Depiction of the direction of median principal compressibility (green arrow) in γ -levofloxacin; (b) depiction of the impact of increasing β -angle in the unit cell of γ -levofloxacin; (c) depiction of the voids in γ -levofloxacin at ambient pressure (LICWOM) (Mercury void analysis using 0.6 Å probe radius and a grid spacing of 0.2 Å).

Despite the minimal compression of the a -axis, there is a discontinuity in the length indicating a slight expansion at 1.14 GPa. The shear implied by the change to the β -angle has implications for the intermolecular interactions particularly reducing the fluorine...fluorine interactions which has been shown to be a destabilising interaction (Figure 3.7b)⁷⁰. An increase in the a -axis will, to some degree, mitigate against the increase in repulsion as the material is compressed.

Both the O and LH studies have benefitted from the use of single crystal diffraction in addition to powder diffraction to provide a more detailed understanding of the

changes that occur in the systems and elucidate the changes to the intermolecular interactions. The principal axes of strain in O and LH follow similar directions to L γ where the most compressible direction is perpendicular to the stacking of the molecules, followed by the interlayer direction, then along the chain of the molecules as exemplified by O (Figure 3.8; LH Figure 3.9). Across the different solid forms, the energy between the stacked molecules contributes substantially to the overall energy of the systems. LH has the strongest interaction energies (*ca.* -90 kJ mol⁻¹) with O and L γ possessing weaker interactions (*ca.* -70 kJ mol⁻¹ and *ca.* -50 kJ mol⁻¹). The response of these interactions to compression are in stark contrast to one another where the dimers in O become repulsive at a greater rate than those in LH.

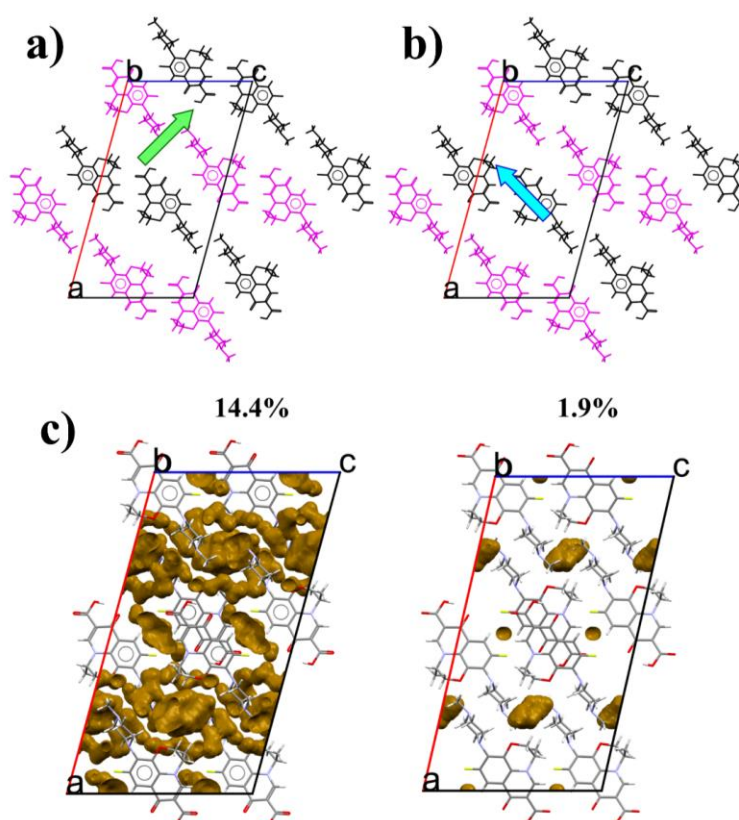


Figure 3.8. (Top) Depictions of the directions of (a) median (green arrow) and (b) minimum (blue arrow) principal compressibilities in O. The black and pink molecular colouring indicates that the ‘rows’ of molecules are tilted at a 23° angle relative to one another. (Bottom) c) Depiction of the voids in O at ambient pressure (left; CUYCEF) and 5.20 GPa (right) (Mercury void analysis using 0.6 Å probe radius and a grid spacing of 0.2 Å).

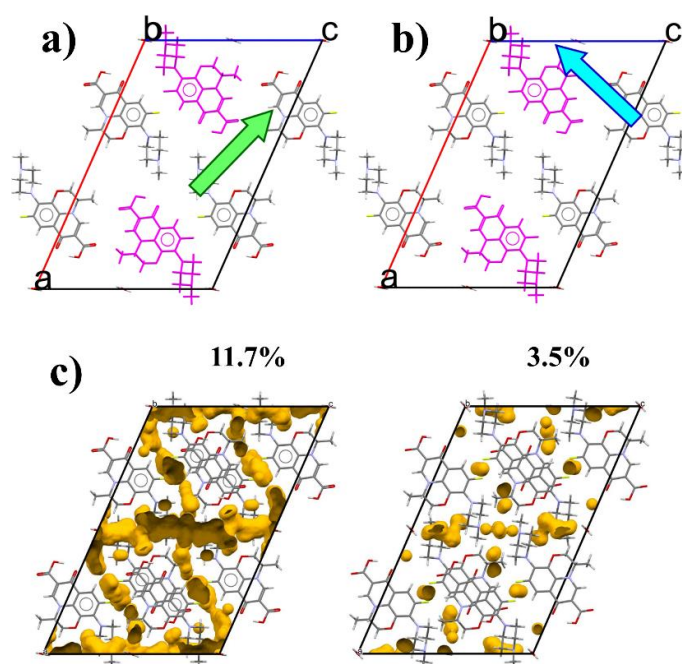


Figure 3.9. (Top) Depictions of the directions of (a) median (green arrow) and (b) minimum (blue arrow) principal compressibilities in levofloxacin hemihydrate. The neutral and pink molecular colouring indicates the different conformers in the unit cell. (Bottom) c) Depiction of voids in levofloxacin hemihydrate at ambient pressure (left; YUJNUM01) and 2.27 GPa (right) (Mercury void analysis using 0.6 Å probe radius and a grid spacing of 0.2 Å).

The response in the median and minimum direction is where the solids differentiate themselves. Neighbouring molecules in $L\gamma$ lie in the same plane as one another, while in the O and LH structures the neighbouring columns in the ac -plane are either tilted (23°) or offset from one another, respectively. This difference in the packing impacts the compression characteristics. The tilted interaction in O provides a greater ability to absorb the compression hence a difference in the eigenvalues is observed between the median and minimum compression. This is shown by the invariant behaviour of the intermolecular energy between the molecules (Interaction 1) in comparison to those interactions where the molecules are parallel which rapidly become less stable (Interactions 2 and 3) (Figure 3.10). In LH, the neighbouring molecules are offset along the b -axis (column direction), creating pseudolayers where molecules can overlap and reduce side-by-side repulsion, especially from equatorial methyl groups,

giving a shallow energetic response to compression (LH; Interaction 5). The planar interaction in O reduces significantly from *ca.* 3.4 Å to 2.95 Å (above) and 2.88 Å (below) up to 5 GPa. The observations show a much larger reduction compared with a recent study of benzo[a]pyrene, where the observed intermolecular values in this study equate to *ca.* 12 GPa in benzo[a]pyrene⁸². This is a notable result as benzo[a]pyrene is a planar molecule and one would anticipate that the interactions perpendicular to the molecules would be rather soft in character hence follow a similar compression rate to O. Benzo[a]pyrene is a purely carbon based molecule whereas the heteroatomic nature of the backbone of O provides an overall more attractive interaction. At 12 GPa, the benzo[a] pyrene π -interaction has significant contributions from the electrostatic, polarisation and dispersion terms that are outweighed by the repulsion between the molecules making the overall interaction unattractive (*ca.* 9 kJ mol⁻¹); the extent of the repulsion energy is twice that of O, indicating that the heteroatomic nature of the O backbone is key to the extended compression.

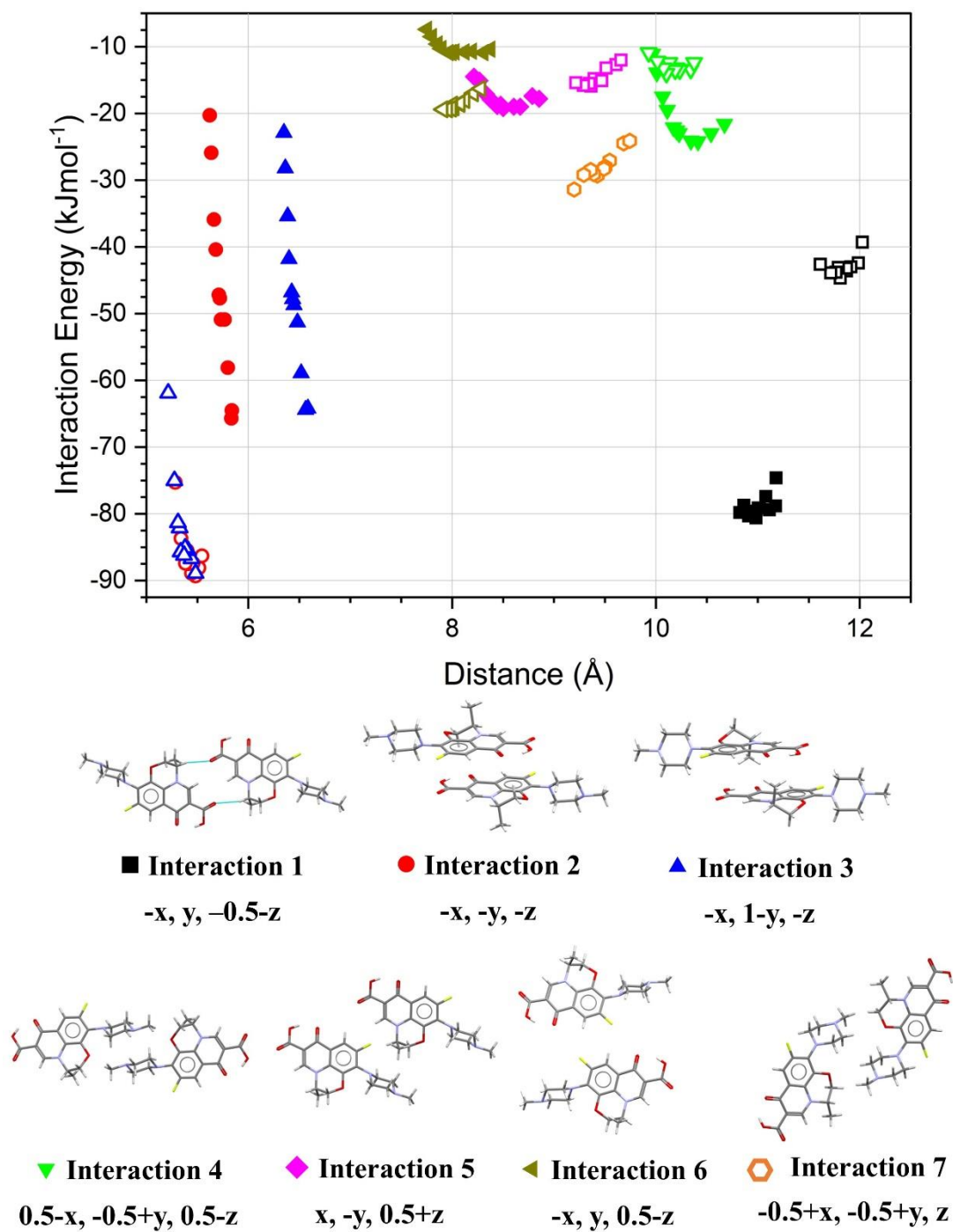


Figure 3.10. The six most influential intermolecular interactions between two molecules in O (solid symbols) and equivalent interactions in LH (open symbols), determined by PIXEL. (Top) Graph displaying the total interaction energies plotted against the distance between the centres of geometry of the interacting molecules as a function of pressure. (Bottom) Interactions in O; the energies for LH are based on similar interactions.

The interaction energies presented correspond to equivalent interactions in both the O and LH phases. Notably, interaction 1 in the O phase exhibits a significantly lower energy while maintaining a similar invariance to pressure that can be attributed to an offset of the molecules perpendicular to the molecular plane. Stacking interactions (Interaction 2 and 3) are markedly stronger in the LH phase, occurring at distances 1.5–2 Å shorter than in the O phase. The impact of pressure on the stacking interactions is common across the two solids with a rapid destabilisation on compression. The interaction involving the piperazine ring (Interaction 4) is similar in orientation, but the attractive contributions to energy are far greater in O than LH, although this is coupled with a commensurate increase in repulsion. Due to the symmetry, O features two equivalent Interaction 4s, while this interaction is split into Interaction 4 and Interaction 7 in LH, with the latter becoming more stable than observed in O.

3.4.3. Potential phase transformations under hydrostatic pressure

3.4.3.1 γ -Levofloxacin

From the initial XRPD studies only Ly showed a potential phase transition at 1.14 GPa where there is a small discontinuity in the molecular volume (Figure 3.6) using the unit cell parameters of the low-pressure phase. The diffraction patterns of Ly compressed in a methanol-ethanol mixture indicates that there is a peak on the side of the prominent (001) reflection indicating that the cell may have changed hence the description of the structure using the ambient pressure unit cell may not be reflective of the true change (Figure 3.11, Figure A3.18). Attempts at indexing this new phase were unsuccessful due to the potential of mixed phases in the system. Subsequent loading in petroleum ether indicated that the sample had transformed and was predominantly LH (Figure 3.11b).

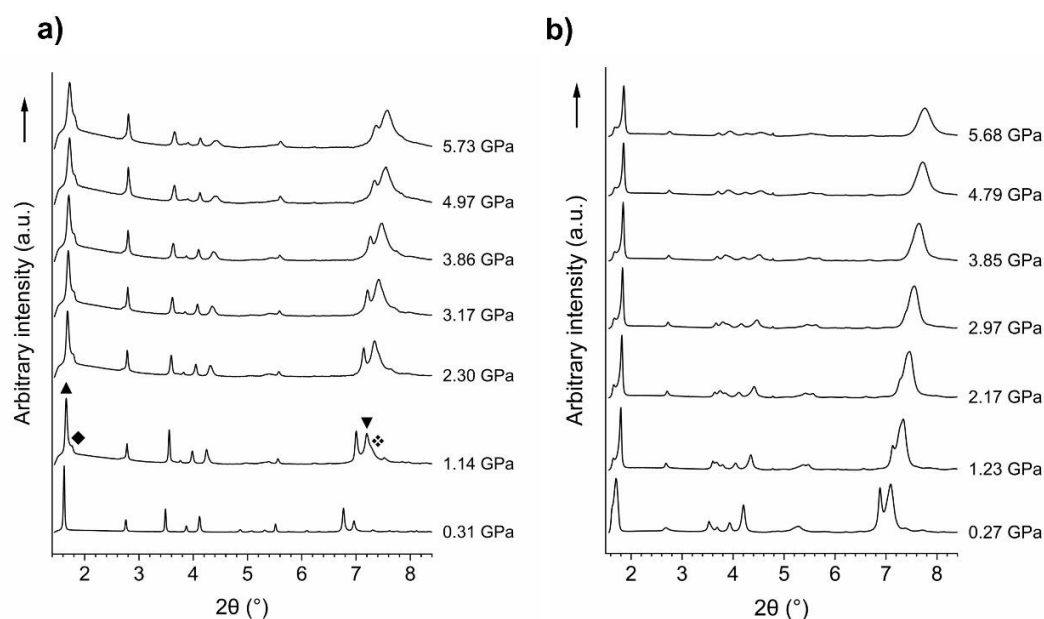


Figure 3.11. Powder patterns during compression of γ -levofloxacin in (a) methanol-ethanol mixture and (b) petroleum ether. In (a), symbols indicate: $\blacktriangle$ (001), $\blacklozenge$ shoulder to (001) (corresponding to levofloxacin hemihydrate's (200) reflection), $\blacktriangledown$ (021), $\blacklozenge$ shoulder to (021) (corresponding to levofloxacin hemihydrate's (021) reflection). In (b), the sample in petroleum ether appears to have transformed and is predominantly levofloxacin hemihydrate. The 2 θ positions of the most intense peak below 2° and its shoulder do not match $\blacktriangle$ and $\blacklozenge$. Peaks between 3.5-4° and around 7° indicate that there is a substantial proportion of levofloxacin hemihydrate present. The patterns have been included for completeness to show the study was conducted but unsuccessful.

3.4.3.2 Levofloxacin hemihydrate

During one of the compression experiments of the single crystal of LH in methanol-ethanol a significant change occurred to the crystal (Figure 3.12). The crystal was initially cut with a scalpel along its longest axis before being loaded into a DAC. Unexpectedly, upon loading, the crystal's dimensions changed from $131\text{ }\mu\text{m} \times 125\text{ }\mu\text{m}$ to $179\text{ }\mu\text{m} \times 91\text{ }\mu\text{m}$. It is noted that the unit cell parameters extracted from the XRPD measurements indicate a slight increase in the volume which has been observed previously in selenourea that may indicate a far greater involvement of the solvent that may facilitate the change⁸³. As the pressure increased, the crystal's dimensions shrunk to $151\text{ }\mu\text{m} \times 86\text{ }\mu\text{m}$ at 1.68 GPa, with its physical appearance deteriorating as the edges became serrated and uneven. In general, the solubility of APIs decreases with pressure; however, anecdotal evidence from other systems suggests that the solubility can increase, presumably due to the solid phase becoming more thermodynamically unstable at higher pressures. Here, the reduction in size of crystal is observed, which could be due to its dissolution under pressure given LH is slightly soluble in methanol and ethanol^{72,73} followed by the crystallisation of a more stable phase at 2.27 GPa two days after compression.

As the new phase developed there was a decrease in pressure to 2.18 GPa and after nine days the pressure inside the DAC had further reduced to 1.85 GPa. Raman spectroscopy analysis of the original crystal (Figure 3.12; region 3) and the crystallised material (regions 1 and 2) revealed distinct phonon regions for each, indicating change in solid form. The larger region 3 is the single crystal from which the diffraction data indicate are of the compressed original phase. This is the only loading where this

behaviour was observed. Further attempts to repeat this experiment as a single crystal and the powder diffraction experiments showed no changes to the solid form.

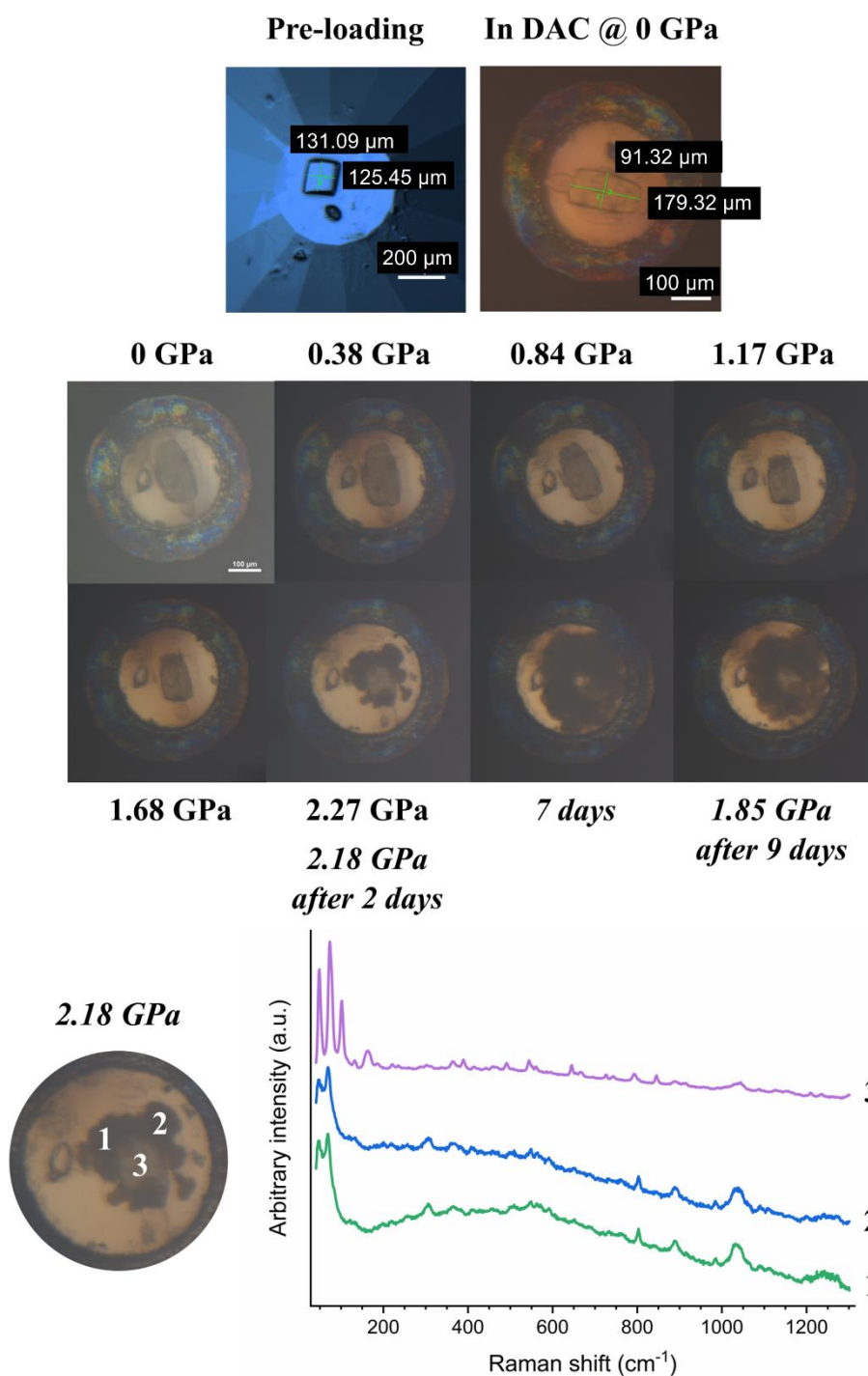


Figure 3.12. Images showing the change in dimensions of a single crystal of LH compressed in a methanol-ethanol mixture. The bottom graph displays the Raman spectra collected from the numbered areas in the sample chamber shown to the left.

A key consideration from these studies is that the water content of the media appears to play a crucial role in enabling the retention of the hydrated form. Repeated experiments conducted on a single crystal using a fresh standard 4:1 methanol-ethanol mixture would result in the crystal deteriorating over the course of an hour becoming opaque and losing its single crystal diffraction. However, using 16:3:1 methanol-ethanol-water prevents this deterioration occurring which would enable single crystal data to be collected; only one data collection was collected to prove the single crystal diffraction was possible. During this process another unsuccessful loading (no ruby) indicated the crystal remained intact. So, it seems that there is a distinct water content that may be required to induce the phase transition in LH, but this has not been investigated any further.

It is pertinent to note that the change in behaviour with the powder (Figure 3.13) and single crystal can also be impacted by two further factors: supercompression beyond transition point, and the solid-to-PTM ratio. Supercompression involves the compression of a phase past its thermodynamically stable region and into a metastable state where it is trapped and kinetically stable. This type of behaviour has been reported in high pressure studies of various organic systems, such as L-serine Form I¹⁷, γ -glycine²⁰, and others⁸⁴. A further source of difference was that measurements in the XRPD study were collected at a faster rate *ca.* 3 min per pressure point for synchrotron powder measurement compared with half a day for in-house single-crystal measurement, plus a one to two day wait between measurements. The ratio of solid to solvent can be a factor where dissolution mediated transitions may be suppressed if there is too little liquid present.

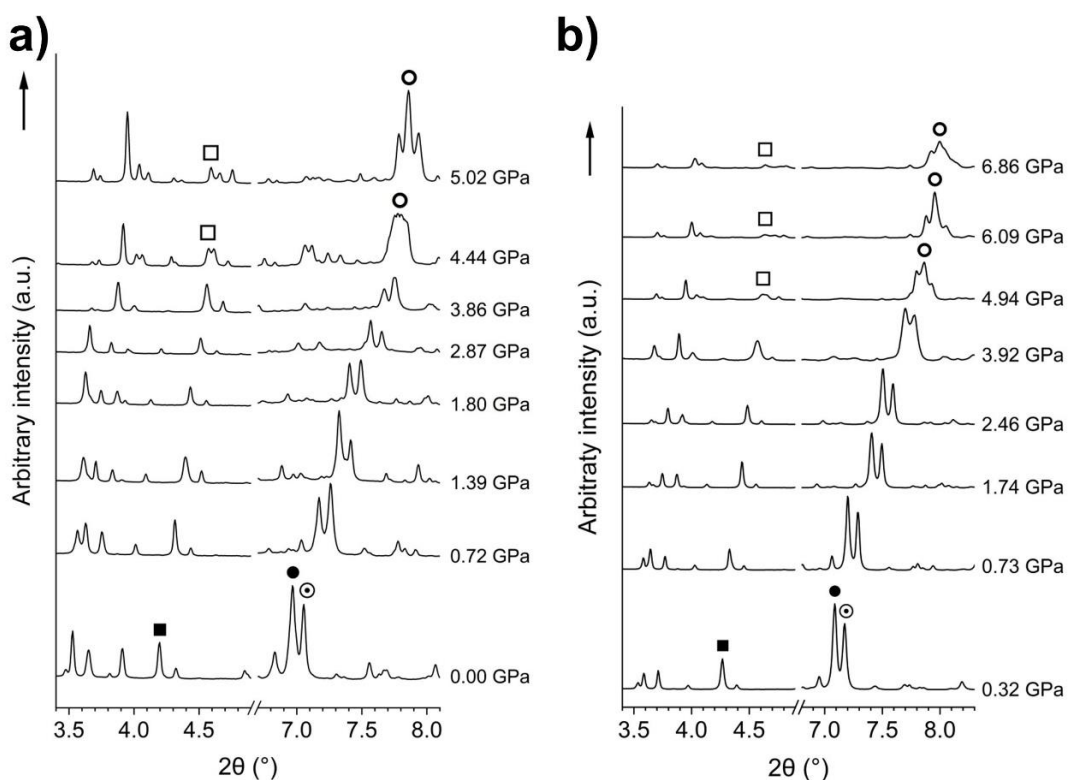


Figure 3.13. Powder patterns during compression of levofloxacin hemihydrate in (a) methanol-ethanol mixture and (b) petroleum ether. Symbols indicate: ■ ($3\bar{1}1$), □ new peak neighbouring ($3\bar{1}1$), ● (021), ⊙ ($22\bar{1}$), ● new peak nestled between (021) and ($22\bar{1}$).

The powder diffraction studies showed that there was a phase transition at higher pressures (3.86 GPa) due to the appearance of two new peaks, one neighbouring the ($3\bar{1}1$) reflection and another between the (021) and ($22\bar{1}$) reflections (Figure 3.13). To identify this change, SC-XRD was performed with petroleum ether as the pressure transmitting medium to mitigate against any chance of the reconstructive transition observed previously. While there was no change observed at 3.86 GPa there was one identified between 4.05 and 5.16 GPa.

Following the phase transition there is a reduction in symmetry from $C2$ to $P1$ where all the molecules become independent from one another, i.e. four independent molecules of levofloxacin and two molecules of water. There is a slight reduction in

the unit cell volume compared with the reduced cell volume of phase I. The only clear change to the structure is the increase in the disorder around the oxazino ring where the C–O (C65–O16) group takes two positions (60:40 occupancy). The overlay of the structures onto the high-pressure unit cell indicates the small shift in the overall positions of molecules with respect to each other (Figure 3.14). Only two data collections were obtained above the transition; only one of these provided data of sufficient quality for structural refinement (5.97 GPa).

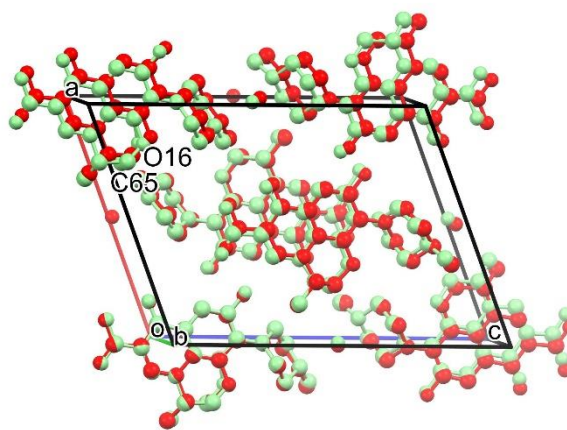


Figure 3.14. Overlay of the new high-pressure phase of LH (light green) with the low-pressure phase prior to the transition (red) indicating the similarity in structure.

3.4.3.3 Ofloxacin

O is not immune to phase transitions either, despite the observations from the initial investigation with the single crystal. The powder patterns of O show new peaks in the powder diffraction pattern (shoulder on the $(20\bar{2})$ reflection and shoulder on the (202) reflection (Figure 3.15)) above 4.65 GPa in the methanol-ethanol PTM. Pawley and Rietveld fits to the patterns above the transition confirm that the shoulders arise from a new phase (Figure A3.20). This change was not initially observed in the single crystal study up to the maximum pressure applied (5.2 GPa), hence particle size may be a factor in the observation of the phase. The phase transition is reversible to the ambient pressure form at 1.65 GPa (Figure 3.15b). There is a suggestion that this also occurs in the purely compressed material (Figure A3.19a) but not in petroleum ether, indicating that the medium has an impact on the polymorphic conversion. The potential phase transformation is likely driven by the interactions between the stacked molecules becoming increasingly repulsive upon compression. Rapid increases in molecular dimer energies have explained pressure-driven transitions in other organic systems, for example, salicylaldoxime and hydroquinone–methanol/acetonitrile clathrates^{85,86}, where the interactions become sufficiently destabilising to drive transformation to another crystalline form. The raw diffraction images before and after the transition reveal that the powder has become more even, indicating that the particles have decreased in size, through either a mechanical breakage of the crystals or a reconstructive transition (Figure A3.21).

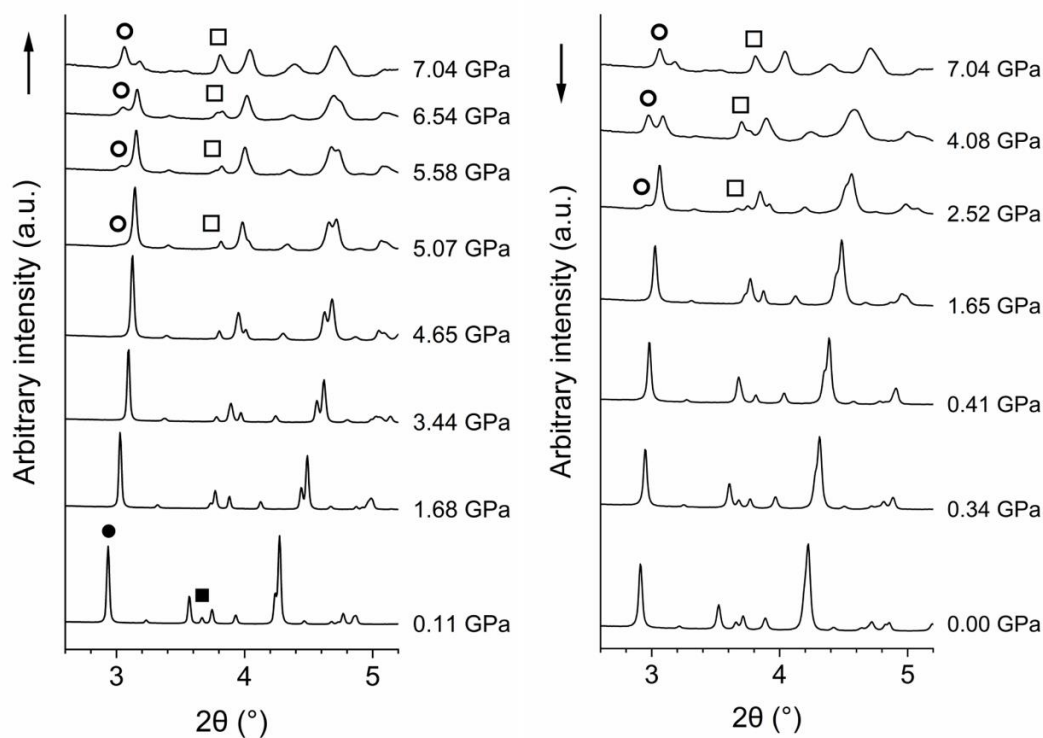


Figure 3.15. Powder patterns during the compression and subsequent decompression of ofloxacin in a methanol-ethanol mixture. During compression, a potential phase transition is observed between 4.65 and 5.07 GPa. Symbols indicate: ● ($20\bar{2}$), ○ new peak next to ($20\bar{2}$), ■ (202), □ new peak next to (202).

The compression of the pure samples without the pressure transmitting medium indicates that whilst there is a general broadening of the sample, it does remain crystalline (Figure A3.19). The ofloxacin does appear to undergo the phase transition at approximately a similar pressure whether hydrostatic or non-hydrostatic which indicates that it is a volume decrease effect rather than a result of shearing of the solids.

3.5. Conclusions

This study provides a comprehensive high-pressure crystallographic analysis of the *RS*- and *S*-forms of 9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid, specifically ofloxacin, γ -levofloxacin and levofloxacin hemihydrate. All three forms display notable compressibility and softness, attributed to the predominance of dispersive intermolecular interactions and parallel molecular packing motifs. While the compression in different media was overall not different, the choice of pressure-transmitting medium as well as particle size can facilitate phase transitions. This is most apparent in the 4:1 methanol-ethanol mixture where there is some degree of solubility.

Distinct pressure-induced phase transitions were observed across the forms, with γ -levofloxacin exhibiting a notably low transition pressure (*ca.* 1.14 GPa), and levofloxacin hemihydrate demonstrating sensitivity to the water content of the pressure-transmitting medium, undergoing a reconstructive transition only under specific conditions. Inert medium compression further revealed levofloxacin hemihydrate's structural persistence up to *ca.* 5.1 GPa before transitioning to a lower-symmetry polymorph. In ofloxacin, high-pressure X-ray powder diffraction suggested a subtle phase transition above 4.65 GPa, although this was not confirmed via single-crystal data.

Together, these results underscore the structural diversity and mechanical responsiveness of these fluoroquinolone derivatives under pressure. The interplay between stereochemistry, hydration state, and external pressure highlights key

considerations for solid form selection in pharmaceutical development. These findings also emphasise the value of combined hydrostatic and non-hydrostatic pressure studies in understanding phase behaviour relevant to drug formulation, manufacturing, and long-term stability.

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Appendix

Each of the solids was characterised prior to the pressure study to ensure the purity of the samples before the synchrotron experiments. The Pawley fits to the data indicate that the solids were a pure phase. Details of the data collection are in the main paper. The unit cell parameters were taken from the Cambridge Structural Database entries for ofloxacin (CUYCEF), levofloxacin hemihydrate (YUJNUM01) and γ -levofloxacin (LICWOM).

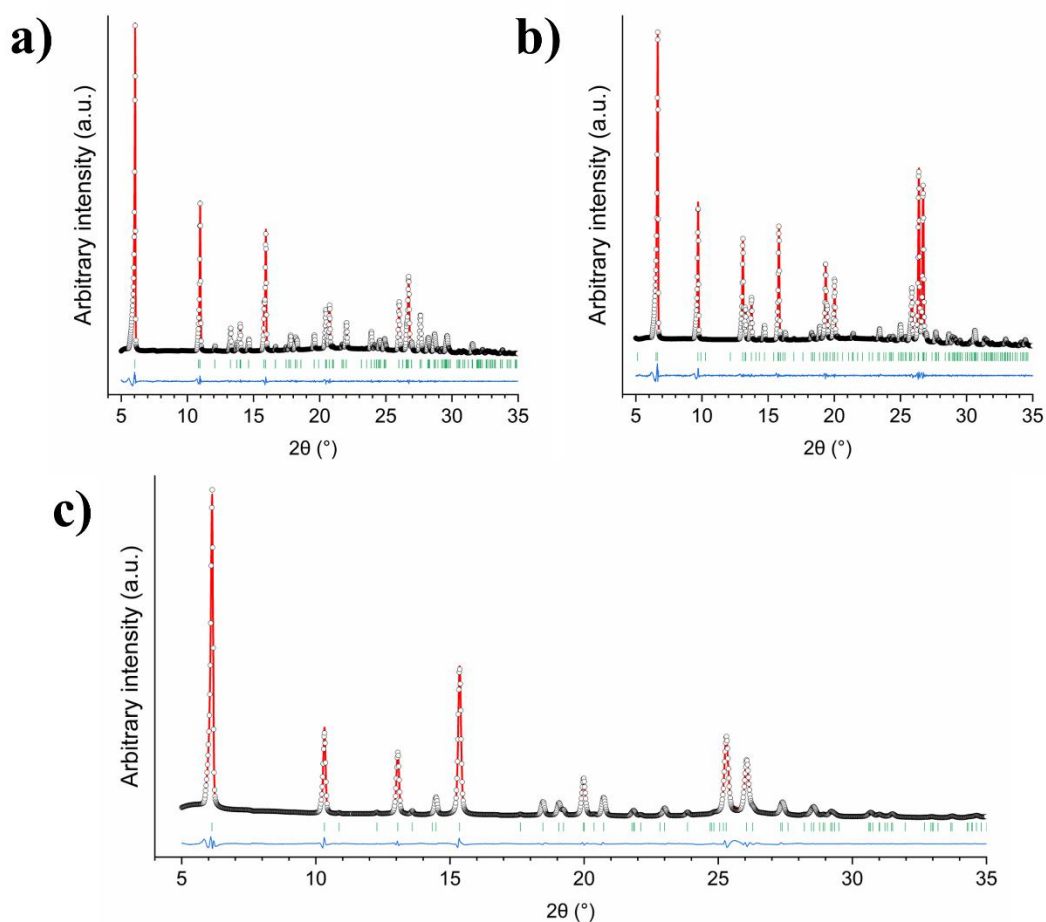


Figure A3.16. Pawley refinement of the powder patterns of (a) ofloxacin, (b) levofloxacin hemihydrate and (c) γ -levofloxacin collected in-house at ambient conditions.

Table A3.3. Details on the crystal structure and experimental temperature of the CSD entries for ofloxacin, γ -levofloxacin and levofloxacin hemihydrate.

	Space group	a (Å)	b (Å)	c (Å)	β (°)	Volume (Å ³)	Molecular volume (Å ³)	Z'	Temperature (K)
Ofloxacin CUYCEF ²⁷	C2/c	30.322(4)	6.8607(8)	16.9199(16)	105.271(11)	3395.6(7)	424.446	1	293
γ-Levofloxacin LICWOM ³²	C2	17.2739(10)	7.0307(2)	15.4605(8)	104.204(5)	1820.24(16)	455.060	1	423
Levofloxacin hemihydrate YUJNUM01 ³⁰	C2	28.7580(8)	6.7988(2)	18.7648(6)	113.847(3)	3355.67(17)	419.459	2	296
Reduced Unit cell parameters									
	a (Å)	b (Å)	c (Å)	α (°)	β (°)	γ (°)	Vol (Å ³)		
Ofloxacin CUYCEF ²⁷	6.861	15.544	16.920	104.89	90	102.75	1697.782		
γ-Levofloxacin LICWOM ³²	7.031	9.325	15.460	103.14	90	112.15	1677.837		
Levofloxacin hemihydrate YUJNUM01 ³⁰	6.799	14.775	18.765	113.17	90	103.30	1677.837		

Table A3.4. Experimental details

For all structures: Chemical formula: $C_{18}H_{20}FN_3O_4$, monoclinic, $C2/c$, $Z = 8$ with $Mr = 361.37$. Experiments were carried out at 296 K with Mo $K\alpha$ radiation. Refinement was on 238 parameters with 248 restraints. H-atom parameters were constrained.

	OFLO_AMBIE NT	OFLO_0p28	OFLO_0p76	OFLO_1p24
Crystal data				
a, b, c (Å)	30.251 (10), 6.8318 (8), 16.845 (2)	29.962 (13), 6.6982 (11), 16.738 (3)	29.630 (7), 6.5439 (6), 16.5404 (16)	29.425 (10), 6.4513 (8), 16.439 (2)
β (°)	105.15 (2)	104.82 (3)	104.354 (16)	104.14 (2)
V (Å ³)	3360.3 (12)	3247.3 (16)	3107.0 (9)	3026.0 (12)
μ (mm ⁻¹)	0.11	0.11	0.12	0.12
Crystal size (mm)	$0.2 \times 0.05 \times 0.05$	$0.2 \times 0.05 \times 0.05$	$0.2 \times 0.05 \times 0.05$	$0.2 \times 0.05 \times 0.05$
Data collection				
Absorption correction	Multi-scan	Multi-scan	Multi-scan	Multi-scan
$T_{\min}, T_{\max}$	0.625, 0.745	0.659, 0.745	0.671, 0.745	0.672, 0.745
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	5571, 826, 450	3332, 803, 430	5492, 792, 493	5594, 773, 486
R_{int}	0.079	0.064	0.073	0.071
$\theta_{\max}$ (°)	23.3	23.2	23.3	23.3
$(\sin \theta/l)_{\max}$ (Å ⁻¹)	0.557	0.554	0.556	0.556
Refinement				
$R[F^2 > 2\sigma(F^2)],$ $wR(F^2), S$	0.042, 0.106, 1.03	0.043, 0.105, 1.03	0.042, 0.115, 1.15	0.049, 0.134, 1.11

No. of reflections	826	803	792	773
	$w = 1/[\sigma^2(F_o^2) + (0.0428P)^2 + 2.724P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.049P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0594P)^2 + 0.4936P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0386P)^2 + 15.8378P]$ where $P = (F_o^2 + 2F_c^2)/3$
$\rho_{\max}, \rho_{\min}$ (e Å ⁻³)	0.08, -0.09	0.10, -0.09	0.10, -0.10	0.14, -0.17

	OFLO_1p81	OFLO-2p16	OFLO_2p47	OFLO_3p0
Crystal data				
a, b, c (Å)	29.123 (7), 6.3326 (6), 16.2627 (16)	29.051 (7), 6.2990 (6), 16.2226 (17)	28.984 (8), 6.2603 (6), 16.1666 (16)	28.803 (7), 6.1832 (5), 16.0548 (14)
β (°)	103.781 (17)	103.694 (16)	103.600 (18)	103.372 (15)
V (Å ³)	2912.9 (9)	2884.3 (9)	2851.2 (9)	2781.8 (7)
μ (mm ⁻¹)	0.13	0.13	0.13	0.13
Crystal size (mm)	0.2 × 0.05 × 0.05	0.2 × 0.05 × 0.05	0.2 × 0.05 × 0.05	0.2 × 0.05 × 0.05
Data collection				
Absorption correction	Multi-scan	Multi-scan	Multi-scan	Multi-scan
$T_{\min}, T_{\max}$	0.665, 0.745	0.677, 0.745	0.670, 0.745	0.681, 0.745
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	5261, 737, 490	4013, 719, 486	5267, 714, 495	5369, 692, 497
R_{int}	0.064	0.060	0.067	0.061
$\theta_{\max}$ (°)	23.3	23.3	23.3	23.3
$(\sin \theta/l)_{\max}$ (Å ⁻¹)	0.557	0.557	0.556	0.556

Refinement				
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.045, 0.127, 1.12	0.042, 0.117, 1.11	0.039, 0.079, 1.15	0.036, 0.082, 1.10
No. of reflections	737	719	714	692
	$w = 1/[\sigma^2(F_o^2) + (0.0763P)^2 + 1.4703P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0649P)^2 + 1.7593P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0201P)^2 + 5.3119P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0216P)^2 + 7.593P]$ where $P = (F_o^2 + 2F_c^2)/3$
$\rho_{\max}$, $\rho_{\min}$ (e Å ⁻³)	0.19, -0.14	0.12, -0.11	0.15, -0.13	0.12, -0.12

	OFLO_3p62	OFLO_4p47	OFLO_5p2
Crystal data			
a , b , c (Å)	28.697 (7), 6.1346 (5), 15.9813 (14)	28.547 (7), 6.0686 (5), 15.8759 (14)	28.443 (9), 6.0167 (7), 15.7781 (17)
β (°)	103.278 (15)	103.121 (16)	102.951 (18)
V (Å ³)	2738.2 (7)	2678.6 (8)	2631.5 (10)
μ (mm ⁻¹)	0.13	0.14	0.14
Crystal size (mm)	0.2 × 0.05 × 0.05	0.2 × 0.05 × 0.05	0.2 × 0.05 × 0.05
Data collection			
Absorption correction	Multi-scan	Multi-scan	Multi-scan
$T_{\min}$, $T_{\max}$	0.646, 0.745	0.679, 0.745	0.669, 0.745
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	4764, 686, 483	4915, 672, 478	3791, 666, 469

R_{int}	0.060	0.061	0.057
$\theta_{\text{max}} (^{\circ})$	23.3	23.3	23.3
$(\sin \theta/\lambda)_{\text{max}} (\text{\AA}^{-1})$	0.556	0.556	0.556
Refinement			
$R[F^2 > 2\sigma(F^2)],$ $wR(F^2), S$	0.038, 0.097, 1.11	0.038, 0.093, 1.10	0.031, 0.084, 1.05
No. of reflections	686	672	666
	$w = 1/[\sigma^2(F_o^2) + (0.0446P)^2 + 4.3269P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0282P)^2 + 9.9383P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0534P)^2 + 0.3586P]$ where $P = (F_o^2 + 2F_c^2)/3$
$\rho_{\text{max}}, \rho_{\text{min}} (\text{e \AA}^{-3})$	0.15, -0.14	0.13, -0.14	0.10, -0.13

Table A3.5. Experimental details

For all structures: Chemical formula: $C_{18}H_{20}FN_3O_4 \cdot 0.5(H_2O)$, monoclinic, $C2$, $Z = 8$ with $M_r = 370.38$. Experiments were carried out at 298 K with Mo $K\alpha$ radiation. Refinement was on 225 parameters with 123 restraints. H-atom parameters were constrained.

	LH_ME_0p38	LH_ME_0p84	LH_ME_1p17	LH_ME_1p68
Crystal data				
a, b, c (Å)	28.512 (5), 6.7261 (4), 18.5315 (13)	28.224 (4), 6.6179 (4), 18.353 (1)	28.099 (5), 6.5600 (4), 18.2689 (11)	27.928 (5), 6.4713 (4), 18.1487 (13)
β (°)	113.557 (12)	113.462 (9)	113.434 (10)	113.447 (12)
V (Å ³)	3257.7 (7)	3144.6 (6)	3089.7 (6)	3009.2 (7)
μ (mm ⁻¹)	0.12	0.12	0.12	0.13
Crystal size (mm)	0.18 × 0.09 × 0.02	0.18 × 0.09 × 0.02	0.18 × 0.09 × 0.02	0.18 × 0.09 × 0.02
Data collection				
Absorption correction	Multi-scan	Multi-scan	Multi-scan	Multi-scan
$T_{\min}, T_{\max}$	0.668, 0.745	0.668, 0.745	0.668, 0.745	0.668, 0.745
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	7699, 1529, 1116	7420, 1472, 1134	7304, 1447, 1151	7133, 1399, 1128
R_{int}	0.046	0.044	0.044	0.045
$\theta_{\max}$ (°)	23.3	23.4	23.4	23.3
$(\sin \theta/l)_{\max}$ (Å ⁻¹)	0.557	0.558	0.558	0.556
Refinement				
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.080, 0.259, 1.07	0.070, 0.209, 1.08	0.065, 0.197, 1.07	0.060, 0.178, 1.04

No. of reflections	1529	1472	1447	1399
$\rho_{\max}, \rho_{\min}$ (e Å ⁻³)	0.28, -0.33	0.21, -0.25	0.21, -0.22	0.20, -0.22
Absolute structure	Flack x determined using 393 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).	Flack x determined using 403 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).	Flack x determined using 423 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).	Flack x determined using 406 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	0.7 (10)	-0.1 (10)	-1.0 (10)	-1.1 (10)

	LH_ME_2p27
Crystal data	
a, b, c (Å)	27.806 (6), 6.4028 (5), 18.0642 (15)
β (°)	113.520 (14)
V (Å ³)	2948.9 (8)
μ (mm ⁻¹)	0.13
Crystal size (mm)	0.18 × 0.09 × 0.02
Data collection	
Absorption correction	Multi-scan
$T_{\min}, T_{\max}$	0.668, 0.745
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	6966, 1377, 1115

R_{int}	0.044
θ_{max} (°)	23.3
$(\sin \theta/l)_{\text{max}}$ (Å ⁻¹)	0.557
Refinement	
$R[F^2 > 2\sigma(F^2)],$ $wR(F^2), S$	0.056, 0.149, 1.05
No. of reflections	1377
$\rho_{\text{max}}, \rho_{\text{min}}$ (e Å ⁻³)	0.19, -0.22
Absolute structure	Flack x determined using 405 quotients $[(I^+)-(I^-)]/[(I^+)+(I^-)]$ (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	-0.4 (10)

Table A3.6. Experimental details

For most structures: Chemical formula: $C_{18}H_{20}FN_3O_4 \cdot 0.5(H_2O)$, monoclinic, $C2$, $Z = 8$ with $M_r = 370.38$. The highest pressure structure is observed in triclinic $P1$. Experiments were carried out at 298 K with Mo $K\alpha$ radiation. Refinement was on 225 parameters with 123 restraints for low-pressure phase and 396 parameters with 114 restraints for the high-pressure phase. H-atom parameters were constrained.

	LH_PE_0p29	LH_PE_1p26	LH_PE_2p19	LH_PE_2p4
Crystal data				
a, b, c (Å)	28.753 (4), 6.7900 (8), 18.636 (5)	28.1035 (17), 6.5548 (4), 18.263 (2)	27.878 (3), 6.4360 (6), 18.107 (3)	27.834 (2), 6.4010 (5), 18.072 (3)
β (°)	113.838 (11)	113.490 (4)	113.447 (8)	113.524 (5)
V (Å ³)	3328.0 (10)	3085.5 (5)	2980.5 (7)	2952.2 (5)
Z	8	8	8	8
μ (mm ⁻¹)	0.12	0.12	0.13	0.13
Crystal size (mm)	$0.18 \times 0.09 \times$ 0.02	$0.18 \times 0.09 \times$ 0.02	$0.18 \times 0.09 \times$ 0.02	$0.18 \times 0.09 \times$ 0.02
Data collection				
Absorption correction	Multi-scan SADABS2016/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.0723 before and 0.0600 after correction. The Ratio of minimum to maximum transmission is 0.8619. The $\lambda/2$	Multi-scan SADABS2016/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.0688 before and 0.0592 after correction. The Ratio of minimum to maximum transmission is 0.8644. The $\lambda/2$	Multi-scan SADABS2016/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.0943 before and 0.0627 after correction. The Ratio of minimum to maximum transmission is 0.8671. The $\lambda/2$	Multi-scan SADABS2016/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.0683 before and 0.0586 after correction. The Ratio of minimum to maximum transmission is 0.8423. The $\lambda/2$

	correction factor is Not present.	correction factor is Not present.	correction factor is Not present.	correction factor is Not present.
Tmin, Tmax	0.642, 0.745	0.644, 0.745	0.646, 0.745	0.628, 0.745
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	14088, 3365, 1849	14280, 3200, 2189	7275, 2495, 1706	15574, 3158, 2411
R_{int}	0.083	0.065	0.058	0.057
θ_{max} (°)	24.8	24.8	23.3	24.7
$(\sin \theta/l)_{\text{max}}$ (Å ⁻¹)	0.590	0.589	0.555	0.588
Refinement				
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.096, 0.323, 1.07	0.079, 0.264, 1.03	0.065, 0.184, 1.03	0.069, 0.197, 1.06
No. of reflections	3365	3200	2495	3158
ρ_{max} , ρ_{min} (e Å ⁻³)	0.52, -0.35	0.54, -0.55	0.27, -0.31	0.40, -0.46
Absolute structure	Flack x determined using 609 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).	Flack x determined using 757 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).	Flack x determined using 560 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).	Flack x determined using 877 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	0.5 (10)	-0.7 (8)	-0.3 (10)	0.1 (6)

	LH_PE_2p99	LH_PE_4p05	LH_PE_5p98
Crystal data			
a, b, c (Å)	27.717 (3), 6.3111 (6), 17.983 (4)	27.562 (3), 6.1945 (7), 17.830 (4)	13.985 (2), 6.0616 (6), 17.534 (3)
α, β, γ (°)	113.633 (9)	113.823 (9)	81.661 (9), 109.861 (12), 79.082 (7)
V (Å ³)	2881.9 (7)	2784.7 (8)	1336.9 (3)
Z	8	8	4
μ (mm ⁻¹)	0.13	0.14	0.14
Crystal size (mm)	0.18 × 0.09 × 0.02	0.18 × 0.09 × 0.02	0.18 × 0.09 × 0.02
Data collection			
Absorption correction	Multi-scan SADABS2016/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.0838 before and 0.0593 after correction. The Ratio of minimum to maximum transmission is 0.847. The $\lambda/2$ correction factor is Not present.	Multi-scan SADABS2016/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.1083 before and 0.0908 after correction. The Ratio of minimum to maximum transmission is 0.837. The $\lambda/2$ correction factor is Not present.	Multi-scan SADABS2016/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.0683 before and 0.0586 after correction. The Ratio of minimum to maximum transmission is 0.8803. The $\lambda/2$ correction factor is Not present.
Tmin, Tmax	0.631, 0.745	0.617, 0.737	0.656, 0.745
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	11056, 3011, 1993	13522, 2926, 1973	7462, 2614, 1587
R_{int}	0.081	0.089	0.057

$\theta_{\max}$ (°)	24.8	24.9	23.3
$(\sin \theta/l)_{\max}$ (Å ⁻¹)	0.590	0.591	0.556
Refinement			
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.073, 0.234, 1.13	0.107, 0.327, 1.20	0.086, 0.276, 1.01
No. of reflections	3011	2926	2614
$\rho_{\max}$, $\rho_{\min}$ (e Å ⁻³)	0.53, -0.41	0.49, -0.62	0.32, -0.36
Absolute structure	Flack x determined using 647 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).	Flack x determined using 637 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).	Flack x determined using 608 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	-0.4 (10)	-1.8 (10)	0.6 (10)

Table A3.7. Refined unit cell parameters for ofloxacin in a methanol-ethanol mixture.

XRPD file number, ESRF	Pressure (GPa)	<i>a</i> -axis (Å)	<i>a</i> -axis error (Å)	<i>b</i> -axis (Å)	<i>b</i> -axis error (Å)	<i>c</i> -axis (Å)	<i>c</i> -axis error (Å)	β -angle (°)	β -angle error (°)	Unit cell volume (Å ³)	Unit cell volume error (Å ³)
In-house XRPD	0.00	30.3166	0.0013	6.8479	0.0001	16.8687	0.0006	105.146	0.003	3380.4	0.2
2	0.11	30.1251	0.0012	6.7686	0.0002	16.7826	0.0007	104.953	0.002	3306.2	0.2
8	0.32	29.9005	0.0012	6.6754	0.0002	16.6813	0.0007	104.733	0.002	3220.1	0.2
13	0.41	29.8275	0.0013	6.6482	0.0002	16.6468	0.0006	104.670	0.002	3193.4	0.2
20	0.46	29.7858	0.0015	6.6379	0.0002	16.6369	0.0007	104.649	0.003	3182.5	0.2
22	0.68	29.6298	0.0014	6.5644	0.0002	16.5510	0.0007	104.456	0.003	3117.3	0.2
26	0.80	29.5676	0.0013	6.5393	0.0002	16.5221	0.0007	104.394	0.003	3094.3	0.2
34	0.95	29.4862	0.0013	6.5100	0.0002	16.4868	0.0008	104.312	0.003	3066.5	0.2
38	1.13	29.3962	0.0015	6.4724	0.0003	16.4395	0.0009	104.214	0.004	3032.1	0.3
43	1.32	29.3325	0.0017	6.4466	0.0004	16.4075	0.0011	104.152	0.004	3008.4	0.3
47	1.57	29.2449	0.0014	6.4087	0.0003	16.3598	0.0008	104.053	0.003	2974.4	0.3
51	1.68	29.2014	0.0018	6.3934	0.0004	16.3375	0.0010	104.000	0.003	2959.5	0.3

55	1.86	29.1421	0.0016	6.3710	0.0003	16.3072	0.0010	103.951	0.003	2938.4	0.3
58	1.99	29.093	0.002	6.3548	0.0004	16.2800	0.0012	103.901	0.004	2921.7	0.4
68	2.10	29.051	0.002	6.3482	0.0004	16.2720	0.0012	103.889	0.004	2913.1	0.3
70	2.28	28.997	0.003	6.3197	0.0006	16.2331	0.0016	103.794	0.005	2888.9	0.5
73	2.52	28.913	0.002	6.2907	0.0004	16.1945	0.0013	103.711	0.004	2861.5	0.4
88	2.70	28.845	0.003	6.2848	0.0003	16.1802	0.0012	103.695	0.005	2849.8	0.4
95	2.99	28.752	0.003	6.2447	0.0005	16.1192	0.0015	103.582	0.006	2813.3	0.5
97	3.44	28.660	0.003	6.1922	0.0004	16.0499	0.0014	103.425	0.006	2770.5	0.4
100	3.81	28.575	0.002	6.1641	0.0003	16.0047	0.0011	103.365	0.006	2742.7	0.3
104	4.12	28.524	0.003	6.1324	0.0003	15.9601	0.0012	103.262	0.006	2717.3	0.3
106	4.65	28.457	0.003	6.0955	0.0003	15.9015	0.0012	103.157	0.006	2685.9	0.4

Table A3.8. Refined unit cell parameters for ofloxacin in petroleum ether.

XRPD file number, ESRF	Pressure (GPa)	<i>a</i> -axis (Å)	<i>a</i> -axis error (Å)	<i>b</i> -axis (Å)	<i>b</i> -axis error (Å)	<i>c</i> -axis (Å)	<i>c</i> -axis error (Å)	β -angle (°)	β -angle error (°)	Unit cell volume (Å ³)	Unit cell volume error (Å ³)
In-house XRPD	0.00	30.3166	0.0013	6.8479	0.0001	16.8687	0.0006	105.146	0.003	3380.4	0.2
7	0.21	29.9815	0.0016	6.7157	0.0003	16.7244	0.0009	104.830	0.003	3255.3	0.3
9	0.34	29.8650	0.0017	6.6654	0.0002	16.6721	0.0008	104.707	0.003	3210.0	0.3
11	0.50	29.7365	0.0019	6.6108	0.0003	16.6075	0.0010	104.565	0.004	3159.8	0.3
14	0.71	29.6066	0.0018	6.5539	0.0002	16.5425	0.0009	104.422	0.004	3108.8	0.3
21	0.99	29.445	0.002	6.4923	0.0003	16.4642	0.0011	104.276	0.005	3050.2	0.3
23	1.38	29.2854	0.0016	6.4173	0.0003	16.3728	0.0009	104.062	0.003	2984.8	0.3
25	1.64	29.1849	0.0016	6.3742	0.0003	16.3158	0.0009	103.949	0.003	2945.7	0.3
27	2.08	29.0473	0.0014	6.3151	0.0002	16.2371	0.0007	103.779	0.003	2892.8	0.2
32	2.34	28.9828	0.0014	6.2883	0.0002	16.1990	0.0008	103.708	0.003	2868.2	0.2
35	2.73	28.8782	0.0016	6.2412	0.0004	16.1302	0.0011	103.583	0.003	2825.9	0.3
42	2.97	28.8043	0.0015	6.2272	0.0002	16.1027	0.0008	103.535	0.003	2808.1	0.2

45	3.25	28.7523	0.0016	6.2030	0.0003	16.0680	0.0008	103.454	0.004	2787.1	0.2
50	3.55	28.6716	0.0018	6.1834	0.0003	16.0331	0.0009	103.405	0.004	2765.0	0.3
52	3.81	28.6190	0.0019	6.1602	0.0003	15.9985	0.0009	103.348	0.004	2744.3	0.3
58	4.05	28.550	0.002	6.1413	0.0003	15.9646	0.0009	103.290	0.004	2724.2	0.3
66	4.39	28.489	0.002	6.1200	0.0003	15.9306	0.0010	103.218	0.005	2704.0	0.3
73	4.69	28.421	0.003	6.0985	0.0003	15.8946	0.0011	103.145	0.006	2682.8	0.4
76	5.59	28.305	0.007	6.0528	0.0006	15.8136	0.0019	102.997	0.010	2639.9	0.8
79	5.96	28.249	0.008	6.0345	0.0006	15.773	0.002	102.963	0.011	2620.4	0.9
80	6.23	28.230	0.010	6.0283	0.0007	15.759	0.002	102.918	0.012	2614.0	1.0

Table A3.9. Refined unit cell parameters for levofloxacin hemihydrate in a methanol-ethanol mixture.

XRPD file number, ESRF	Pressure (GPa)	<i>a</i> -axis (Å)	<i>a</i> -axis error (Å)	<i>b</i> -axis (Å)	<i>b</i> -axis error (Å)	<i>c</i> -axis (Å)	<i>c</i> -axis error (Å)	β -angle (°)	β -angle error (°)	Unit cell volume (Å ³)	Unit cell volume error (Å ³)
In-house XRPD	0	29.1183	0.0015	6.8832	0.0002	18.8462	0.0008	114.066	0.003	3449.0	0.3
5	0	29.190	0.003	6.8948	0.0004	18.8798	0.0019	114.166	0.007	3466.7	0.6
8	0.15	28.937	0.004	6.8357	0.0006	18.759	0.004	113.905	0.012	3392.4	0.9
11	0.45	28.691	0.006	6.7621	0.0011	18.611	0.004	113.725	0.011	3305.5	1.1
14	0.70	28.487	0.003	6.7034	0.0005	18.5060	0.0019	113.587	0.007	3238.7	0.6
17	0.72	28.456	0.003	6.6956	0.0005	18.4970	0.0018	113.591	0.007	3229.7	0.6
23	1.08	28.255	0.003	6.6127	0.0003	18.376	0.002	113.550	0.008	3147.4	0.6
29	1.39	28.114	0.003	6.5531	0.0007	18.2746	0.0017	113.490	0.008	3087.7	0.6
35	1.80	28.033	0.004	6.4860	0.0007	18.203	0.003	113.562	0.009	3033.8	0.7
41	2.22	27.888	0.003	6.4165	0.0004	18.1004	0.0015	113.528	0.007	2969.6	0.5
47	2.87	27.772	0.003	6.3388	0.0005	18.0044	0.0017	113.585	0.008	2904.7	0.5
56	3.21	27.707	0.004	6.3144	0.0006	17.964	0.003	113.553	0.011	2881.1	0.7

59	3.86	27.620	0.005	6.2553	0.0008	17.891	0.003	113.587	0.012	2832.8	0.8
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Table A3.10. Refined unit cell parameters for levofloxacin hemihydrate in petroleum ether.

XRPD file number, ESRF	Pressure (GPa)	<i>a</i> -axis (Å)	<i>a</i> -axis error (Å)	<i>b</i> -axis (Å)	<i>b</i> -axis error (Å)	<i>c</i> -axis (Å)	<i>c</i> -axis error (Å)	β -angle (°)	β -angle error (°)	Unit cell volume (Å ³)	Unit cell volume error (Å ³)
In-house XRPD	0.00	29.1183	0.0015	6.8832	0.0002	18.8462	0.0008	114.066	0.003	3449.0	0.3
5	0.00	29.190	0.003	6.8948	0.0004	18.8798	0.0019	114.166	0.007	3466.7	0.6
2	0.32	28.676	0.005	6.7703	0.0012	18.622	0.004	113.709	0.010	3310.2	1.1
12	0.60	28.404	0.003	6.6871	0.0005	18.455	0.002	113.524	0.006	3214.1	0.5
14	0.73	28.340	0.003	6.6636	0.0005	18.417	0.002	113.503	0.006	3189.5	0.6
23	1.28	28.089	0.004	6.5485	0.0009	18.253	0.002	113.458	0.006	3079.9	0.7
25	1.74	27.948	0.004	6.4745	0.0007	18.150	0.002	113.450	0.007	3012.9	0.7
36	2.26	27.850	0.005	6.4170	0.0011	18.082	0.004	113.475	0.009	2964.1	0.9
41	2.46	27.809	0.004	6.3882	0.0008	18.038	0.003	113.474	0.009	2939.3	0.8
45	3.18	27.676	0.004	6.3078	0.0006	17.939	0.003	113.533	0.009	2871.3	0.7

Table A3.11. Refined unit cell parameters for γ -levofloxacin in a methanol-ethanol mixture.

XRPD file number, ESRF	Pressure (GPa)	<i>a</i> -axis (Å)	<i>a</i> -axis error (Å)	<i>b</i> -axis (Å)	<i>b</i> -axis error (Å)	<i>c</i> -axis (Å)	<i>c</i> -axis error (Å)	β -angle (°)	β -angle error (°)	Unit cell volume (Å ³)	Unit cell volume error (Å ³)
In-house XRPD	0.00	17.503	0.003	7.0367	0.0013	14.720	0.003	101.845	0.008	1774.3	0.6
11	0.31	17.4770	0.0016	6.9550	0.0003	14.850	0.002	102.508	0.008	1762.2	0.3
17	0.41	17.467	0.004	6.9204	0.0011	14.839	0.006	102.74	0.02	1749.5	0.8
24	0.52	17.464	0.003	6.8757	0.0007	14.821	0.004	103.020	0.013	1733.9	0.6
25	0.64	17.451	0.004	6.8336	0.0010	14.782	0.005	103.251	0.015	1715.9	0.7
28	0.75	17.448	0.006	6.8032	0.0017	14.754	0.005	103.421	0.018	1703.6	0.9
33	0.88	17.444	0.006	6.7736	0.0018	14.700	0.005	103.52	0.02	1688.8	0.9
39	1.14	17.520	0.009	6.7312	0.0024	14.708	0.006	103.97	0.02	1686.7	1.2
45	1.32	17.519	0.005	6.7078	0.0011	14.696	0.005	104.00	0.02	1675.6	0.8
48	1.63	17.497	0.004	6.6712	0.0009	14.626	0.005	104.10	0.02	1655.8	0.7
58	1.92	17.518	0.006	6.6431	0.0013	14.571	0.00	104.29	0.02	1643.2	1.1
61	2.30	17.463	0.010	6.597	0.003	14.455	0.008	104.42	0.03	1612.9	1.4

67	2.70	17.479	0.007	6.5697	0.0019	14.416	0.008	104.59	0.02	1602.0	1.2
68	3.17	17.447	0.013	6.537	0.004	14.364	0.009	104.70	0.03	1584.6	1.8
73	3.86	17.45	0.06	6.50	0.02	14.34	0.05	104.91	0.03	1571	9
77	4.33	17.41	0.02	6.467	0.006	14.259	0.015	104.93	0.03	1551	3
88	4.97	17.39	0.03	6.433	0.008	14.20	0.02	105.00	0.03	1534	4
90	5.73	17.38	0.03	6.411	0.009	14.15	0.02	105.07	0.03	1522	4

The data for γ -levofloxacin in petroleum ether was largely the levofloxacin hemihydrate so no parameters determined.

Table A3.12. Table displaying the total lattice energies (E_{tot}) of ofloxacin (CUYCEF) and γ -levofloxacin (LICWOM) at ambient pressure, along with the partitioning into Coulombic energy (E_{Coul}), polarisation energy (E_{pol}), dispersion energy (E_{disp}) and repulsion energy (E_{rep}). All energies are represented in kJ mol^{-1} .

CSD refcode	E_{Coul}	E_{pol}	E_{disp}	E_{rep}	E_{tot}
CUYCEF	-70.9	-28.6	-157.4	101	-156
LICWOM	-65.7	-30.9	-134.2	119	-112

Table A3.13. Fitting parameters of the equations of state for ofloxacin (O), γ -levofloxacin (Ly) and levofloxacin hemihydrate (LH) from pressure studies carried out in a methanol-ethanol mixture (me), petroleum ether (pet) and FluorinertTM FC-70 (FC-70). Powder and single crystal samples were studied using diamond anvil cells and a sapphire capillary cell (SCC). Ly was fitted using the data to 0.88 GPa (before the suspected phase transition). The volumes have been set to be the measured pressures at ambient pressure and fixed based on suggestions of the referee. 2nd-order fits to the powder diffraction studies did not describe the data as well as the 3rd-order with volumes fixed.

	Fitted EoS	V_0	K_0	K'
O me (XRPD) 0-4.65 GPa	3 rd Vinet	3337(11)	8.0(6)	9.3(4)
O pet (XRPD) 0-6.23 GPa	3 rd Vinet	3358(16)	5.9(5)	11.8(4)
O me (SC-XRD) 0-5.20 GPa	3 rd Vinet	3368(24)	6.4(8)	10.0(6)
O SCC FC-70 (SC-XRD) 0.003-0.12 GPa	2 nd Vinet	3379(3)	7.3(6)	1*
LH me (XRPD) 0-3.86 GPa	3 rd Vinet	3490(22)	5.6(8)	11.6(9)
LH pet (XRPD) 0-3.18 GPa	3 rd Vinet	3456(26)	5.4(10)	12.9(12)
LH me (SC-XRD) 0-2.23 GPa	3 rd Vinet	3451	3.6(7)	26(7)

LH SCC FC-70				
(SC-XRD)	2 nd Vinet	3430.6(8)	6.96(17)	1*
0.002-0.12 GPa				
Lγ me (XRPD)	2 nd Vinet	1805(9)	12.6(14)	1*
0-0.88 GPa^{\$}				
Lγ me (XRPD)	3 rd Vinet	1767.5(18)	18.1(11)	9.3(12)
1.1-5.8 GPa***				

* K' is set to 1 in 2nd order Vinet Equation of State hence no error is associated with this value.

\$ The data point at 0.0001 GPa was not used in the fit.

*** 2nd-order fit does not represent the data well. The refinement of all three parameters was unstable. K₀ and K' were refined based on estimated volume of 1766.5. The V₀ was refined with values of K₀ and K' fixed. This was iteratively done. Whilst the errors in the values are likely underestimated, it provides a reasonable fit to the experimental data.

Table A3.14. Principal axes of strain for the crystal structures of ofloxacin (O), γ-levofloxacin (Lγ) and levofloxacin hemihydrate (LH) based on the compression of powder samples in a methanol-ethanol mixture. The table displays the direction of compression and the coefficient of compressibility (K).

PRINCIPAL AXES OF STRAIN					
X1		X2		X3	
Direction	K (TPa ⁻¹)	Direction	K (TPa ⁻¹)	Direction	K (TPa ⁻¹)
O [0,1,0]	23.2(2)	[-0.6082, 0, 0.7938]	15.1(1)	[0.4196, 0, 0.9077]	7.89(7)
Lγ [0,1,0]	17.3(3)	[0.4782, 0, 0.8783]	14.1(6)	[0.8651, 0, -0.5015]	-0.1(6)
LH [0,1,0]	31.4(5)	[0.8115, 0, -0.5844]	16.8(5)	[0.4294, 0, 0.9031]	14.9(2)

Table A3.15. Table displaying the total lattice energies (E_{tot}) of **O** as a function of pressure, along with the lattice energy components: Coulombic energy (E_{Coul}), polarisation energy (E_{pol}), dispersion energy (E_{disp}) and repulsion energy (E_{rep}). All energies are represented in kJ mol^{-1} . E_{tot} is corrected by considering the changes to the internal molecular energy. Pixel provides the values based on the intermolecular interactions and does not consider any change to the conformation of the molecule and the energy change that may arise from this change. The initial Gaussian calculation provides this, and the relative energies (from the ambient form) can be calculated and summed with the E_{tot} from Pixel.

Pressure (GPa)	E_{Coul}	E_{pol}	E_{disp}	E_{rep}	E_{tot}	E_{tot} corrected
CUYCEF	-70.9	-30.8	-157.4	101	-156	-156
0	-64.5	-38.1	-162.8	109.1	-149	-172.8
0.28	-72.8	-45.7	-181	138.6	-153.3	-168.0
0.76	-91	-53.7	-208.3	193	-152	-186.9
1.24	-102.4	-65.5	-228.2	238.4	-145.9	-180.3
1.81	-123.8	-69.6	-253.3	298.9	-143.7	-168.7
2.16	-130.5	-74.1	-261.8	320.8	-141.1	-167.8
2.47	-140.4	-85.7	-270.3	344.6	-140.1	-165.7
3	-161.5	-96.1	-290.7	408.5	-129.4	-154.4
3.62	-175.1	-109.2	-304.8	454.9	-121.1	-144.2
4.47	-195.8	-119.5	-323.5	524.2	-104.4	-124.2
5.2	-214.9	-115.4	-340.1	581.3	-93.2	-107.6

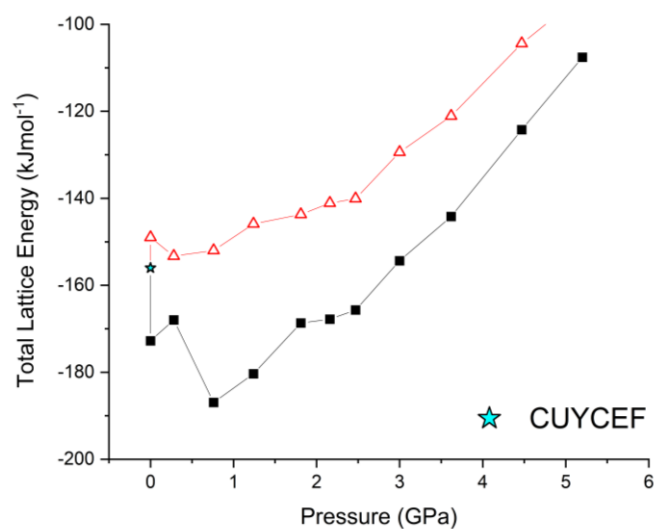


Figure A3.17. Total lattice energies of ofloxacin as calculated by PIXEL (red triangles) and total lattice energies of ofloxacin adjusted for the molecular energy due to conformational changes (black squares) relative to the ambient pressure configuration (CUYCEF).

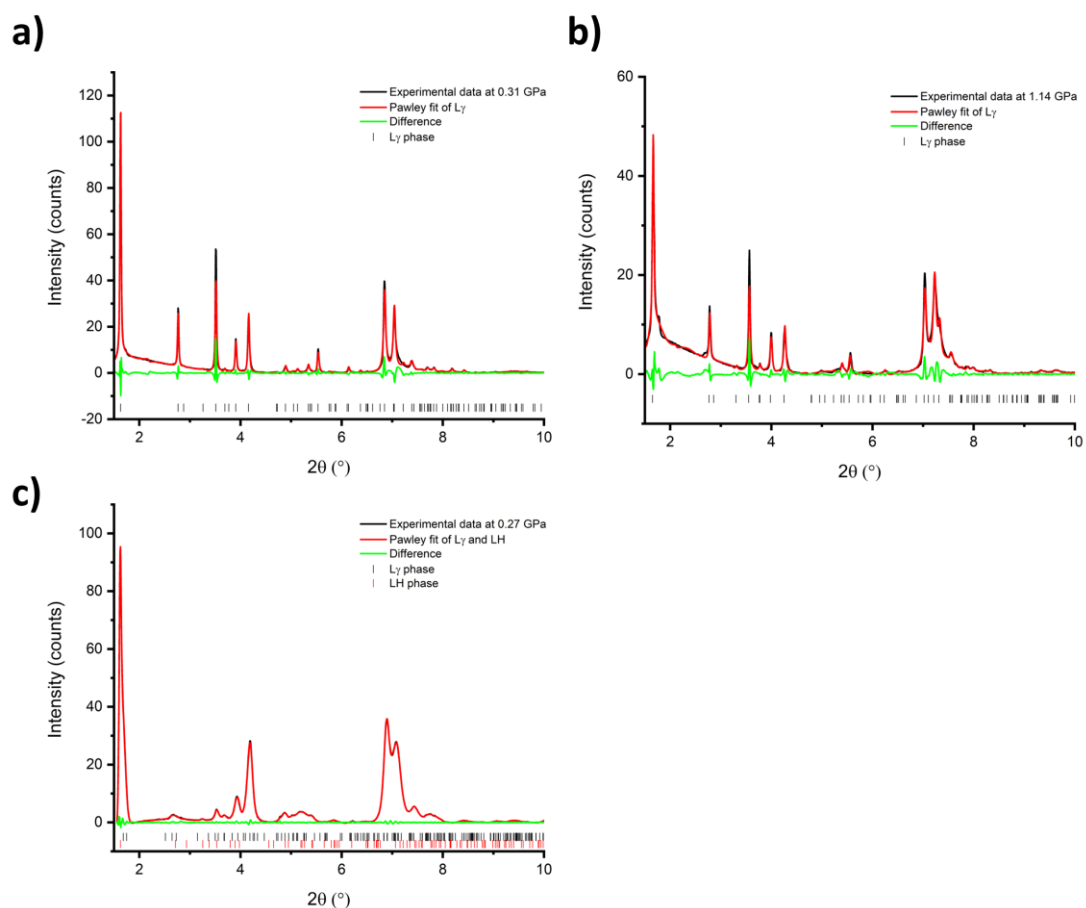


Figure A3.18. (a,b) Pawley fits to the data for γ -levofloxacin before and after transition indicating the misfits around the (001) reflection; (c) Pawley fit of the γ -levofloxacin in petroleum ether indicating the mixed phase with LH.

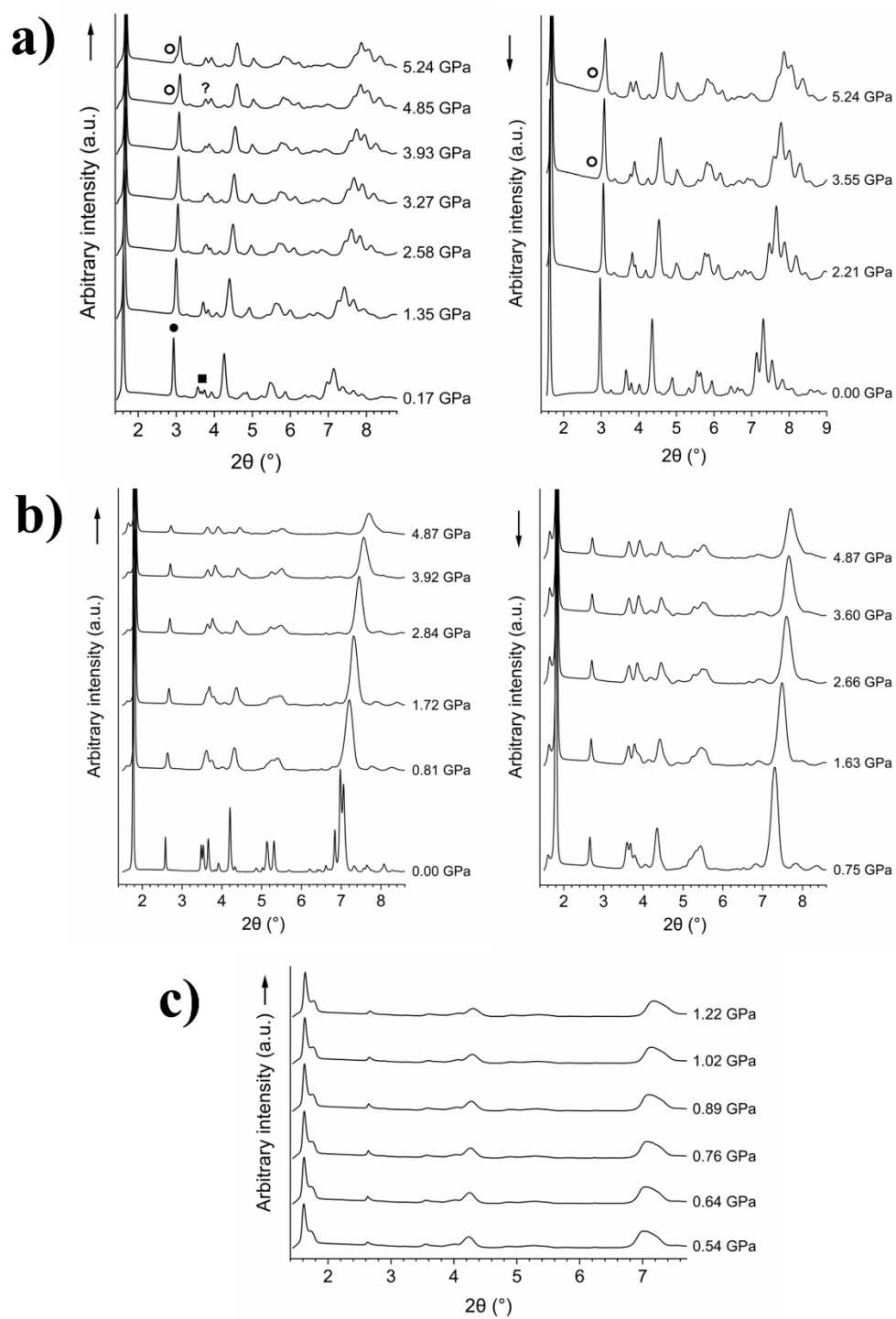


Figure A3.19. Powder patterns during compression and decompression of (a) ofloxacin, (b) levofloxacin hemihydrate and (c) γ -levofloxacin without a pressure-transmitting medium. In (a), symbols indicate: $\bullet$ (202), $\circ$ new shoulder on (202), $\blacksquare$ (202), ? no clear distinction of a new shoulder on (202).

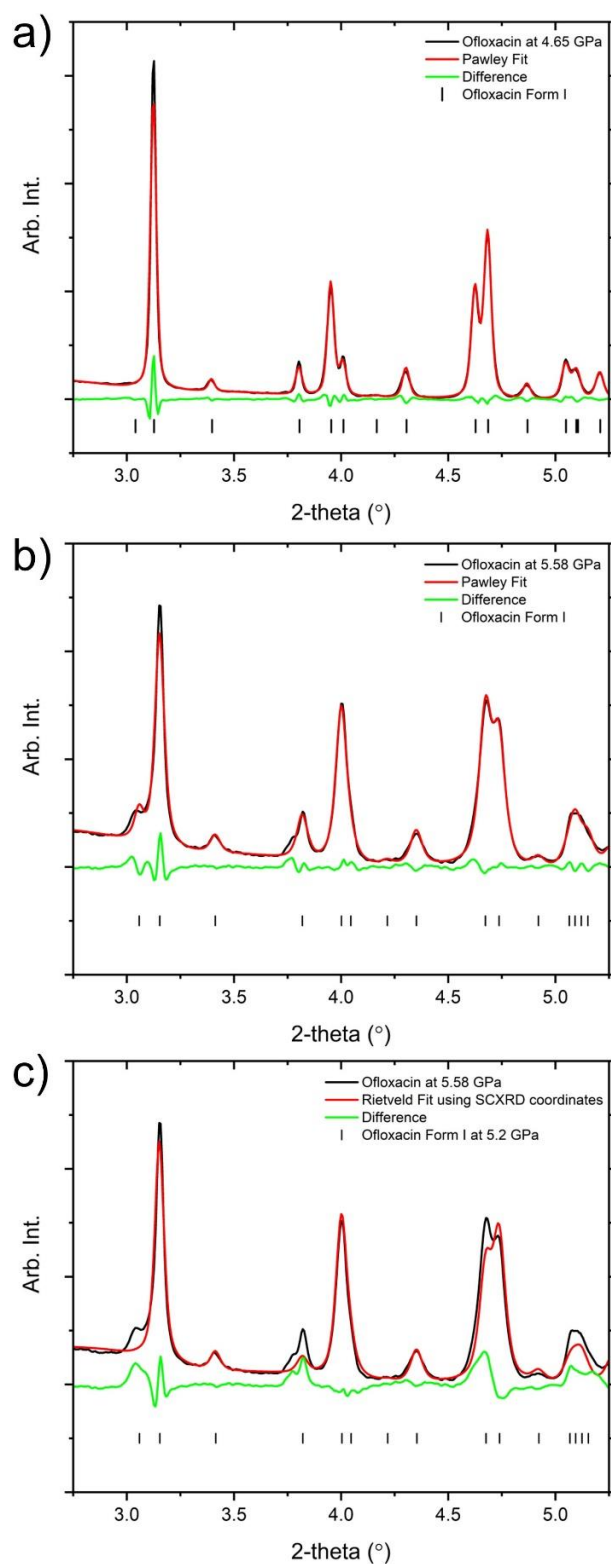
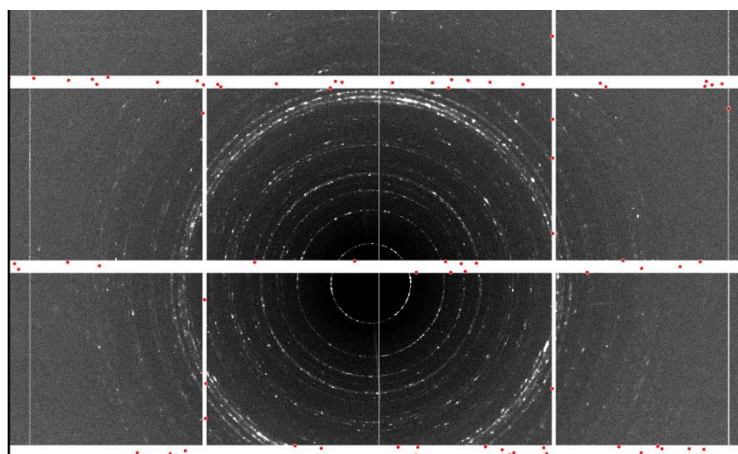
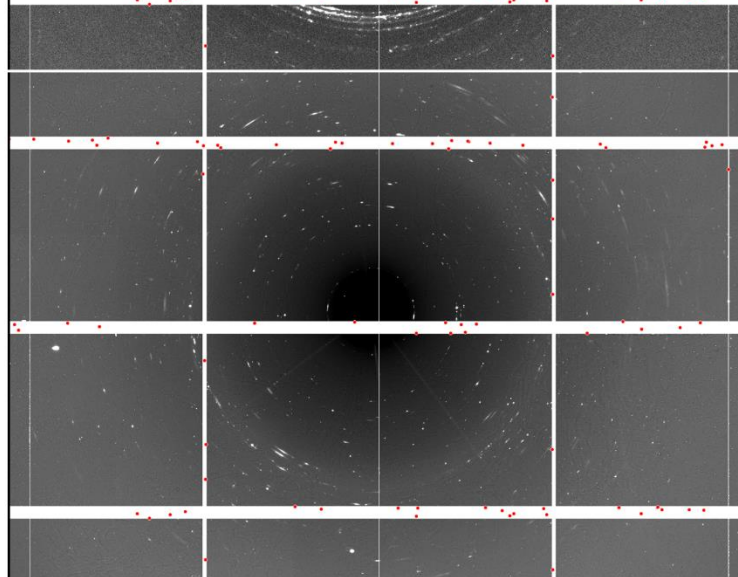


Figure A3.20. Fits to the ofloxacin data (a) below the transition and (b,c) above the transition in methanol-ethanol mixture. Both the Pawley and Rietveld fit are present for powder patterns above the transition to demonstrate that there is a new phase appearing with shoulder on the (20 $\bar{2}$) and (202) reflections (3.06 and 3.77°). The coordinates of the structure determination at 5.2 GPa are used refining the unit cell and scale factor and preferred orientation only. The Pawley fit uses the intensities of the pattern hence fits the peak at 3.06° but this intensity is not related to a change in the atomic positions.

a) Ofloxacin



b) Levofloxacin
hemihydrate



c) γ -levofloxacin

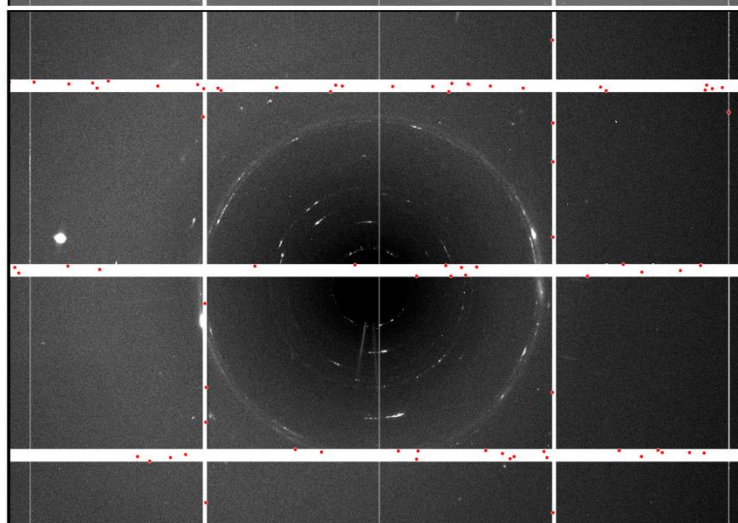


Figure A3.21. Raw images of the diffraction for (a) ofloxacin, (b) levofloxacin hemihydrate and (c) γ -levofloxacin in methanol-ethanol, indicating the poor powder averaging for the sample.

Chapter 4 - Analysis of pressure-induced molecular displacement in pharmaceutical crystals

4.1. Abstract

The mechanical behaviour of pharmaceutical solids can be rationalised by examining how their structures respond to applied pressure, and high-pressure crystallography provides detailed information on this structural response. However, conventional analyses used to interpret these data, such as principal axes of strain, describe the overall structural response of the unit cell to pressure and are constrained by crystallographic symmetry, meaning they do not explicitly capture how molecules move relative to one another and miss subtle molecular rearrangements. This Chapter presents a new method and accompanying tool, MolDis, for the direct visualisation and examination of pressure-induced molecular displacement in crystalline materials. The method uses sequential high-pressure single-crystal X-ray diffraction data to calculate displacement vectors for molecules within the unit cell relative to a reference molecule, determining both the magnitude and direction of molecular translation.

The method is validated using paracetamol Forms I and II, for which the molecular displacement captured is consistent with previously reported pressure-induced structural responses. MolDis is subsequently applied to a set of structurally related acetanilides (4-chloroacetanilide, 4-methylacetanilide and metacetamol) for which the high-pressure behaviour is unknown. Across all systems, molecular displacements largely preserve the ambient packing, with corrugated chains and layers flattening under pressure. When combined with intermolecular interaction energies, void space and slip plane analyses, MolDis captures molecular displacements normal to potential

slip planes and elucidates the governing influences of each translation. The additional insight gained by explicitly tracking molecular translation provides a deeper understanding of pressure-induced structural change.

4.2. Introduction

During the development and manufacture of solid oral dosage forms, the mechanical behaviour of an active pharmaceutical ingredient (API) is characterised at an early stage to inform the selection of excipients and manufacturing processes, and to mitigate any undesirable behaviour^{1,2}. The mechanical properties of an API have an impact on processing steps such as milling³ and tableting^{4,5}, and may give rise to issues such as punch sticking⁶. Several studies have demonstrated that crystal engineering strategies can be successfully employed to address these challenges⁷⁻⁹. From these studies, general structural trends can be identified, but a reliable basis for accurately predicting how a structure will respond to applied stress in different scenarios remains limited.

A crystal may display varying mechanical behaviour under different conditions, with the nature and extent of the response influenced by the direction of applied stress^{10,11}, the magnitude of applied stress (e.g., elastic versus plastic response¹²) and the particle size of the crystal (e.g., plastic versus brittle response¹³). Mechanical responses are broadly classified as either disintegrative or restorative¹⁴. Disintegrative effects, such as fragmentation, occur after an induction period under the applied external stimulus (e.g., stress), and before the crystal relaxes. Restorative effects arise once the external stimulus is removed, allowing the crystal to relax and reorganise its structure to relieve deformation; they are further categorised as elastic or plastic depending on whether the crystal fully or only partially regains its original shape. Plastic deformation, elastic deformation and brittle fracture are common types of deformation observed in the development and manufacturing of pharmaceutical solid oral dosage forms.

The mechanical behaviour of a crystal can be rationalised by examining how its structure responds to applied pressure. Examining pressure-induced structural changes enables macroscopic mechanical behaviour to be related to the underlying molecular packing and intermolecular interactions^{15,16}. Understanding the impact of pressure on APIs is also important, as it has been shown to induce changes in the molecular packing of pharmaceutically relevant crystalline solids (e.g., changes in relative position, molecular orientation, conformation, and intermolecular contacts)^{17,18}. Pressure-induced molecular rearrangements may preserve the original phase, lead to a phase transition, or result in a loss of crystallinity through amorphisation¹⁸. Understanding how and why molecules reorganise within the crystal structure under pressure is necessary to interpret pressure-induced structural behaviour and its relationship to mechanical response.

High-pressure X-ray diffraction is a powerful experimental technique for determining the structure of crystalline materials at discrete pressure points and accessing this molecular-level response. Structural response to pressure can be analysed using quantitative parameters such as unit-cell compressibility, principal strains and bulk modulus^{19,20}, as well as changes to intermolecular interactions²¹. Complementary particle informatics approaches can extend this analysis further by identifying features such as void space and potential slip planes in the structure^{22,23}. While these analyses, in combination, provide a detailed understanding of how the crystal structure and the relationships between molecules within it change as a function of pressure, they do not directly represent how individual molecules move relative to one another within the unit cell. Changes in the relative positions of neighbouring molecules are inferred from structural changes rather than explicitly determined.

Additionally, the inferred molecular displacements are inherently constrained by crystallographic symmetry and the symmetry elements present in the unit cell. As a result, how pressure is accommodated at a molecular scale, including the direction and magnitude of molecular displacement, remains difficult to interpret from conventional analyses alone.

To address this limitation, this Chapter presents a molecular displacement analysis tool, MolDis, that explicitly tracks how molecules translate relative to a reference molecule within the unit cell across a series of data collections. MolDis quantifies the relative direction and magnitude of molecular displacement, and its output is visualised using the CCDC software Mercury²⁴. This tool has been developed for high-pressure research but could equally be used for other sequential structural studies of molecules under external stimulus, e.g., variable-temperature studies²⁵. By representing these changes as displacement vectors derived from crystallographic data, the approach provides a direct and intuitive visualisation of molecular translation under hydrostatic compression. To the best of our knowledge, such an approach has not previously been applied to experimental high-pressure crystallographic datasets.

The development and performance of the MolDis displacement analysis tool has used a series of structurally related acetanilides, with paracetamol chosen as the benchmark system. Paracetamol, a widely studied pharmaceutical compound, was selected due to its well-characterised behaviour under pressure at both the molecular and macroscopic levels¹⁸. Paracetamol forms I and II were examined first to validate the displacement analysis against existing knowledge of their structural responses. The analysis was then extended to paracetamol analogues for which the high-pressure behaviour is unknown.

This systematic study explored the influence of subtle steric and electronic modifications on the structural response to pressure. Two para-substituted analogues of paracetamol were studied: 4-chloroacetanilide and 4-methylacetanilide. These compounds share the same molecular core structure as paracetamol but differ in the steric and electronic effects of the small substituent at the para (4') position. Chlorine and the methyl group are volumetrically similar and sterically larger than the hydroxyl group in paracetamol; chlorine is electron-withdrawing, whereas methyl and hydroxyl groups are respectively weakly and strongly electron-donating. Finally, metacetamol, a structural isomer of paracetamol in which the hydroxyl substituent occupies the meta (3') position of the benzene ring, was also studied to assess the influence of substituent positioning on pressure response.

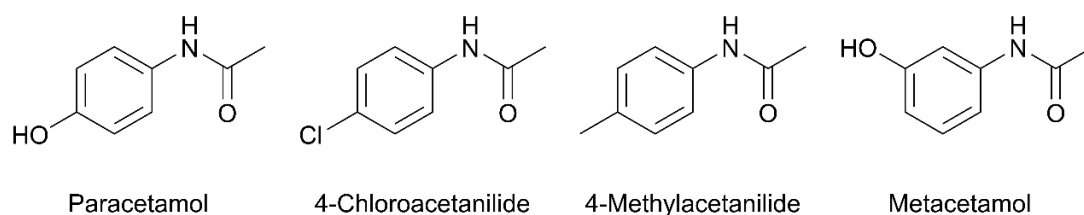


Figure 4.1. Molecular structure of paracetamol, 4-chloroacetanilide, 4-methylacetanilide and metacetamol.

4.3. Materials and Methods

4.3.1. Preparation of single crystals

The chemicals used were purchased from Sigma Aldrich (paracetamol, $\geq 99\%$ purity) and VWR International Ltd. (4-chloroacetanilide, 4-methylacetanilide, metacetamol – all $\geq 98\%$ purity). Analytical reagent-grade ethanol was purchased from Honeywell.

Prism-like crystals of paracetamol Form I were obtained by preparing a saturated solution in ethanol at room temperature and allowing it to evaporate slowly until crystals of suitable size formed²⁶. Single crystals of 4-chloroacetanilide have previously been obtained from an ethanolic solution²⁷. Needle-like crystals of 4-chloroacetanilide were obtained by preparing a saturated solution in ethanol at 40 °C and cooling the solution to room temperature. Cooling crystallisation uses the decrease in solubility with decreasing temperature to generate supersaturation and promote nucleation and crystal growth²⁸.

Single crystals of the monoclinic form of 4-methylacetanilide and metacetamol Form I were prepared using a combined antisolvent and slow evaporation crystallisation method^{29,30}. Water was used as an antisolvent because acetanilides exhibit low solubility in aqueous media, and mixing a second solvent in which the solute is poorly soluble (i.e., antisolvent) induces supersaturation and crystallisation^{31,32}. A room-temperature saturated ethanolic solution was placed in a vial and deionised water was added dropwise until crystals were formed. The solution was agitated by hand between drops to ensure proper mixing. The vial was covered with parafilm and pierced to enable slow evaporation of solution in a fume hood until

the crystals grew to a suitable size for study. Single crystals of paracetamol Form II were prepared following the method reported by Thomas *et al.*³³ and were provided by Dr Michael Devlin (University of Strathclyde).

4.3.2. In-house single-crystal X-ray diffraction at high pressure

High-pressure SC-XRD data were collected for paracetamol Form II, 4-chloroacetanilide, the monoclinic form of 4-methylacetanilide and metacetamol Form I using Merrill-Bassett diamond anvil cells (DACs) equipped with Boehler-Almax design anvils with a 40° opening angle and 600 µm culets. For each sample, a stainless-steel gasket was indented to a ~100 µm thickness, and a 250 µm diameter sample chamber was drilled by spark erosion using an Almax Easylab µDriller. The ruby fluorescence method was used to calibrate the pressure^{34,35}. Petroleum ether (PET 35-60, Alfa Aesar) was used as the pressure-transmitting medium to ensure hydrostatic compression up to 6 GPa³⁶.

SC-XRD was collected at room temperature on a Bruker D8 Venture diffractometer equipped with a 4-circle kappa goniometer and a Photon III C14 CMOS detector and an Incoatec Microfocus Source (IµS Diamond, Mo K α , λ = 0.71073 Å). The pressure was recorded before and after each collection to determine the average pressure experienced throughout the measurement. The pressure limits in this study were dictated by gasket failure in paracetamol Form II and by reaching the hydrostatic limit of PET³⁶ in 4-chloroacetanilide, 4-methylacetanilide and metacetamol. Additionally, the quality of the diffraction data for 4-methylacetanilide and metacetamol worsened at the highest pressures collected.

The diffraction data were processed using the APEX 3 software package. The data were integrated using the dynamic masking procedure in SAINT³⁷, and absorption

corrections were applied using SADABS³⁸. The structures were refined with SHELXL³⁹ in Olex2-1.5⁴⁰ using a full-matrix least-squares on $|F|^2$ refinement. Non-hydrogen atoms were refined anisotropically. RIGU restraints were used where appropriate⁴¹. All hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms.

4.3.3. Synchrotron single-crystal X-ray diffraction at moderate pressure

Data for all systems were collected using the sapphire capillary pressure cell on beamline I19 at the Diamond Light Source (DLS)⁴², following the method described in Chapter 3, Section 3.3.8. The lowest-pressure data point (0.003 GPa; 30 bar) for paracetamol Form I, paracetamol Form II and metacetamol Form I was used as the near-ambient pressure point in the pressure series studied in this Chapter. The diffraction data were processed, integrated and refined following the method described in Section 4.3.2.

4.3.4. Molecular displacement tracking and visualisation

Molecular displacement vectors for each molecule in the unit cell, excluding the reference molecule, were calculated using the computational tool developed in this Chapter and described in Section 4.4.1. The tool was implemented in Python 3.9.⁴³ and made use of the CSD Python API^{44,45} together with the Numpy⁴⁶ library to calculate the direction and magnitude of the molecular displacement. The code is available as open source at <https://github.com/cxb20168/MolDis>. The start and end points of the molecular displacement trajectories were exported in Protein Data Bank (PDB) format. These molecular displacement vectors were overlaid on the crystal structure at the lowest pressure studied and visualised using Mercury 2023.3²⁴.

4.3.5. PIXEL calculations

Intermolecular forces were investigated using the semiempirical PIXEL method developed by Gavezzotti^{47–49}. PIXEL calculations were conducted on high-pressure diffraction data using the MrPIXEL program⁵⁰ through the Mercury graphical interface. Hydrogen positions in the input structures were normalised to conventional neutron values, as the structures were determined by X-ray diffraction. Molecular electron densities were calculated for a cluster of molecules within a 14 Å radius from a central molecule, using Gaussian09W⁵¹ at the B3LYP/6-31G** level of theory⁵² with a condensation level of 4. Symmetry operations between pairs of molecules were visualised in Mercury 2023.3.

4.3.6. Particle Informatics

Molecular and crystal structure analysis was carried out using the CCDC's Mercury v. 2022.3.0 software²⁴. Void space analysis was carried out using the Voids feature in Mercury. The voids were probed using a 0.6 Å probe radius and a grid spacing of *ca.* 0.2 Å. These are non-standard values for this function that allows us to follow changes in the void volume as a function of pressure over the entire pressure range. The surface roughness of molecular planes and the potential slip planes in the systems studied were calculated and visualised using two particle informatics tools included in the CSD Particle suite: Surface Analysis⁵³ and Slip Planes⁵⁴, respectively.

4.3.7. Principal strains

Principal compressibilities and axes of compression were calculated using PASCAL^{20,55}. The principal axes of compression were visualised in VESTA v. 3.5.7⁵⁶ overlaid on a three-dimensional representation of the studied structure.

4.4. Results and Discussion

4.4.1. Design of a method for tracking molecular displacement from relative molecular positions, and a complementary computational tool for its implementation (MolDis)

A new method has been developed to visualise the translation of molecules in a crystalline structure as a function of pressure. This approach provides additional molecular-level insight into the behaviour of organic crystalline materials under compression, based on experimental data. The method calculates molecular displacements in a unit cell relative to a reference molecule and represents these as vectors that can be visualised using Mercury.

The computational tool, named MolDis, was coded in Python using the CSD Python API. The process follows nine steps in which data is extracted, vectors are computed and then visualised. Steps 1-7 describe the data extraction and vector computation, followed by Steps 8-9 for the post-processing and visualisation in Mercury (Figure 4.2).

MolDis requires a set of Crystallographic Information Files (CIFs) from a crystallographic study as input (e.g., a pressure study), in which the atomic labelling is consistent across the dataset. The analysis is limited to a single phase hence studies with phase transitions will require running with any additional phase. To run the Python script, data files must be saved in the same folder and named in a way that sorting them alphabetically or numerically places them in order of increasing pressure.

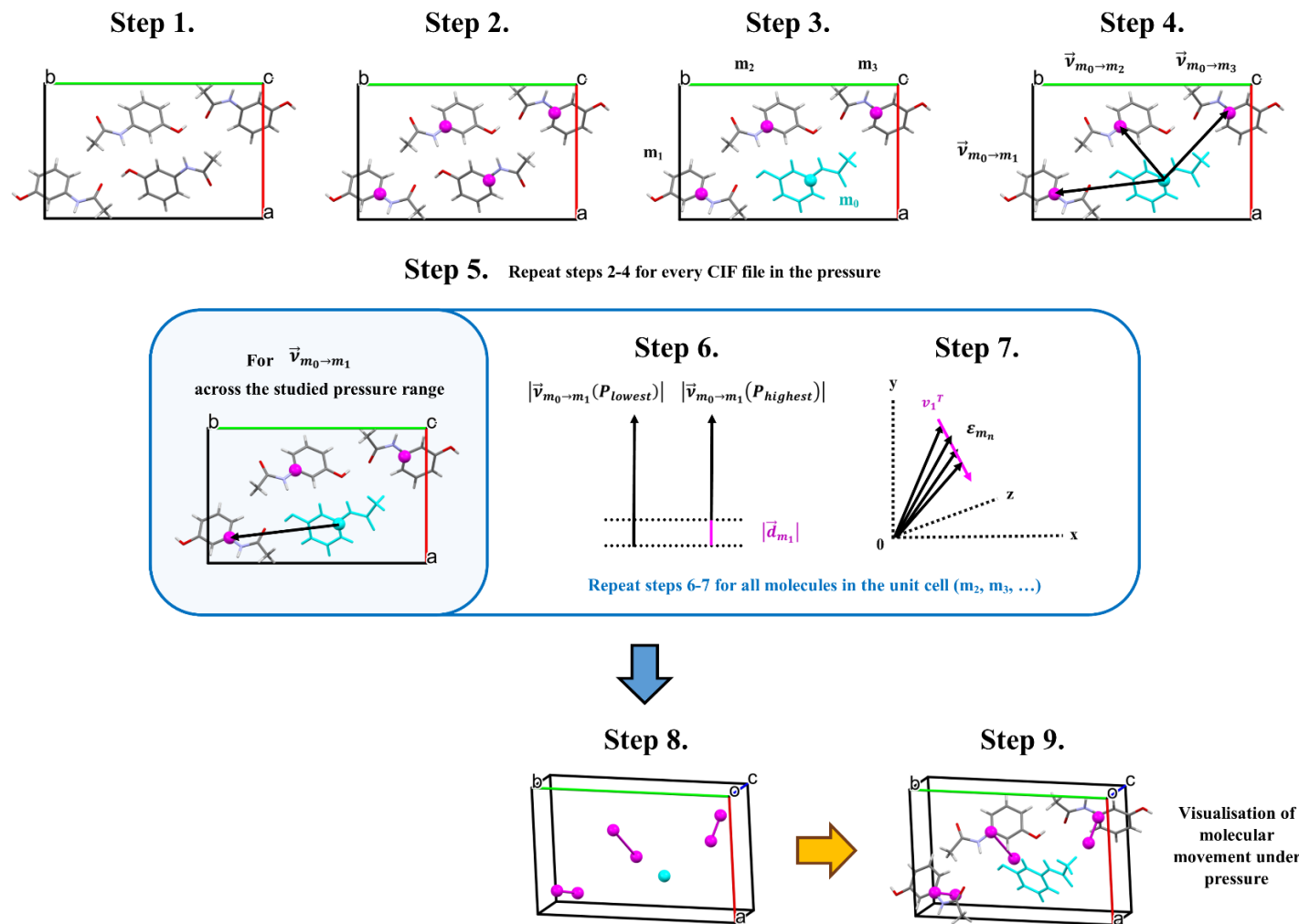


Figure 4.2. Step-by-step diagram illustrating the method developed to track molecular movement in a crystalline structure under an external stimulus. m_n are the molecules in the unit cell (with m_0 being the reference molecule), v is the vector from the molecular centroid of m_0 to that of m_n , and P is the pressure corresponding to a given Crystallographic Information File (CIF).

Step 1: Load and sort CIFs. All CIFs in the specified folder are read and sorted alphabetically and numerically.

Step 2: Calculate molecular centroids. The first CIF in the sorted list is opened, and the centres of geometry for all molecules in the unit cell are calculated (magenta dots in Figure 4.2). If desired, the centre of mass or the aromatic centroid could be chosen however, the centre of geometry is the most representative measure of the central point of the molecule.

Step 3: Output molecular centroid positions. The molecular centroid positions are output in Cartesian coordinates, listed, and stored. The molecule corresponding to the first molecular centroid in the list is arbitrarily selected as the reference molecule m_0 (cyan-colour, Figure 4.2), and will serve as the origin for spatial measurements in the unit cell (Equation 1). Hence it is important that the refined structures use the same coordinates as a starting model. The remaining molecular centroids are labelled $m_1, m_2, \dots, m_n$, in the order they appear in the list (Equation 2). This order is preserved across all CIFs. The Cartesian coordinates of each molecular centroid at a given pressure P are expressed as follows:

$$centroid_{m_0}(P) = (x_0, y_0, z_0) \quad (1)$$

$$centroid_{m_n}(P) = (x_n, y_n, z_n) \quad (2)$$

Step 4: Calculate vectors. The direction (Equation 3) and magnitude (Equation 4) of the vectors from the molecular centroid of m_0 to the other molecular centroids in the unit cell are calculated (black arrows in Figure 4.2).

$$\begin{aligned} \vec{v}_{m_0 \rightarrow m_n}(P) &= centroid_{m_n}(P) - centroid_{m_0}(P) \\ &= [x_n - x_0, y_n - y_0, z_n - z_0] \end{aligned} \quad (3)$$

$$= [m_{n_x}(P), m_{n_y}(P), m_{n_z}(P)]$$

$$|\vec{v}_{m_0 \rightarrow m_n}(P)| = \sqrt{m_{n_x}(P)^2 + m_{n_y}(P)^2 + m_{n_z}(P)^2} \quad (4)$$

Step 5: Repeat across all CIFs. Steps 2-4 are repeated for every CIF in the folder.

For each target molecule m_n , all vectors from m_0 to m_n across the pressure series are collated and stored as a separate group. For example, vectors from m_0 to m_1 across all pressures are stored in a different group from vectors m_0 to m_2 .

Step 6: Calculate the magnitude of molecular displacement. The displacement magnitude of each molecule m_n relative to m_0 across the studied pressure range is calculated by subtracting the vector magnitudes at the highest and lowest pressures (Equation 5). The resulting displacement values are expressed in angstroms (Å).

$$|\vec{d}_{m_n}| = |\vec{v}_{m_0 \rightarrow m_n}(P_{lowest})| - |\vec{v}_{m_0 \rightarrow m_n}(P_{highest})| \quad (5)$$

This step assumes that increasing pressure causes molecules to move closer together, thereby reducing the separation between them.

Step 7: Determine the direction of molecular displacement *via* Principal Component Analysis (PCA). To determine the direction of molecular displacement relative to m_0 across the studied pressure range, a line of best fit is drawn through the endpoints of the vectors from m_0 to each m_n , with all vectors adjusted to start from the origin (0,0,0). This line is obtained using PCA, a statistical technique that identifies the direction of maximum variance in the data. The analysis is performed separately for each vector group created in Step 5.

PCA works by calculating the average point in a group of data points, analysing the relationship between the group's variables in an n-dimensional dataset, and

identifying the direction in which the data points are most spread out. This direction represents the largest variation in the data and highlights the most significant trend. In this case, the data is three-dimensional, using x-, y- and z-coordinates. Details on how PCA is applied in the method are in Steps 7.1-7.7.

Step 7.1: Collect endpoints. From the vectors connecting m_0 to m_n , adjusted to start from a common origin, in this case (0,0,0), the endpoints are grouped into a dataset denoted by ε_{m_n} , where k represents the total number of endpoints (Equation 6).

$$\varepsilon_{m_n} = \{e_1^{(m_n)}, e_2^{(m_n)}, \dots, e_k^{(m_n)}\} \quad (6)$$

Each endpoint $e_i^{(m_n)}$ corresponds to the tip of a vector from m_0 to m_n at any given pressure and is represented as a three-dimensional coordinate vector (Equation 7).

$$e_i^{(m_n)} = \begin{bmatrix} x_i \\ y_i \\ z_i \end{bmatrix} \quad (7)$$

Step 7.2: Calculate the mean point. The mean point μ is calculated from all the endpoints in the dataset ε_{m_n} (Equation 8).

$$\mu = \begin{bmatrix} \bar{x} \\ \bar{y} \\ \bar{z} \end{bmatrix} \quad (8)$$

where:

$$\bar{x} = \frac{1}{k} \sum_{i=1}^k x_i, \bar{y} = \frac{1}{k} \sum_{i=1}^k y_i, \bar{z} = \frac{1}{k} \sum_{i=1}^k z_i$$

This step allows the dataset to be centred before performing PCA. PCA identifies the main direction along which the endpoints are spread. To do this meaningfully, the endpoints must be centred so that the analysis describes how the points are distributed relative to each other (i.e., the variance relative to the mean point). Otherwise, if the endpoints are not centred, the variance is calculated relative to the origin (0,0,0), reflecting the absolute position of the dataset in space rather than how the endpoints are spread.

Step 7.3: Centre the endpoints. The endpoints in the dataset ε_{m_n} are centred around the mean point μ by subtracting μ from each endpoint (Equation 9).

$$e_i^{(m_n)'} = e_i^{(m_n)} - \mu \quad (9)$$

This operation produces a new set of centred endpoints $e_i^{(m_n)'}$, which together form the dataset ε_{m_n}' .

Step 7.4: Construct the covariance matrix. A 3x3 covariance matrix C is constructed from the centred endpoints $e_i^{(m_n)'}$ using Equation 10. This matrix describes the relationships between the x, y and z coordinates, capturing how variations in one dimension relate to variations in the others. $(\cdot)^T$ denotes the transpose of a vector.

$$C = \frac{1}{k-1} \sum_{i=1}^k e_i^{(m_n)'} (e_i^{(m_n)'})^T \quad (10)$$

Step 7.5: Find the eigenvectors and eigenvalues. The eigenvectors v_i and eigenvalues λ_i of the covariance matrix C are obtained by solving the eigenvalue problem shown in Equation 11.

$$Cv_i = \lambda_i v_i \quad (11)$$

In PCA, eigenvectors represent the principal directions along which the data is distributed, while eigenvalues quantify the amount of variance along each direction. A larger eigenvalue indicates that the data points are more widely spread along its corresponding eigenvector.

Step 7.6: Identify the principal component. The eigenvector associated with the largest eigenvalue λ_1 is designated as the principal component v_1 .

v_1 defines the direction of maximum variance in the data and represents the axis that best characterises the three-dimensional distribution of endpoints. Based on this direction, a line of best fit can be defined to describe the displacement of m_n relative to m_0 across the studied pressure range.

Step 7.7: Define the direction of molecular displacement. The line of best fit, denoted as $L(t)$, is expressed parametrically using the mean point μ and the principal component v_1 (Equation 12).

$$L(t) = \mu + tv_1 \quad (12)$$

Here, t is a scalar parameter that determines the position of a point along the line. The direction of the line of best fit is derived from the transpose of the principal component v_1 and expressed as a unit vector (Equation 13).

$$v_1^T = (d_x, d_y, d_z) \quad (13)$$

Although the vector v_1^T defines the direction of molecular displacement (i.e., the line along which displacement occurs), its orientation along this line (whether it points in the $+v_1^T$ or $-v_1^T$ orientation) is ambiguous. It must be

verified to ensure it correctly represents the progression of the endpoints in ε_{m_n} as pressure increases.

Step 8: Prepare a PDB file to visualise molecular displacement.

Once the molecular displacement directions and magnitudes are determined, the next step is to model and visualise this information as a molecular displacement vector for each molecule. For each molecule m_n in a unit cell, a trajectory is drawn starting from its molecular centroid at the lowest pressure studied. To generate this trajectory, the direction vector v_1^T is scaled by ten times the magnitude of the displacement calculated in Step 6. Multiplying the magnitude by a factor of ten helps visualise small displacements in the Mercury visualisation. The endpoint of the trajectory, denoted $e_{model}^{(m_n)}$, is calculated following Equation 14.

$$e_{modelA}^{(m_n)} = centroid_{m_n}(P_{lowest}) + (10 \cdot |\vec{d}_{m_n}|) \cdot v_1^T \quad (14)$$

However, as noted in Step 7.7, the direction vector v_1^T does not specify the sense of displacement. To account for this, a second possible endpoint is calculated by reversing the direction of the vector (Equation 15).

$$e_{modelB}^{(m_n)} = centroid_{m_n}(P_{lowest}) - (10 \cdot |\vec{d}_{m_n}|) \cdot v_1^T \quad (15)$$

Both endpoints are plotted in a three-dimensional graph generated in Origin⁵⁸ and connected to the molecular centroid position at the lowest pressure studied. The dataset of endpoints ε_{m_n} , obtained from the vectors connecting m_0 to m_n across the pressure range studied, is also plotted. The correct sense of displacement is determined by visually assessing which trajectory best aligns with the spread of endpoint data (Figure 4.3).

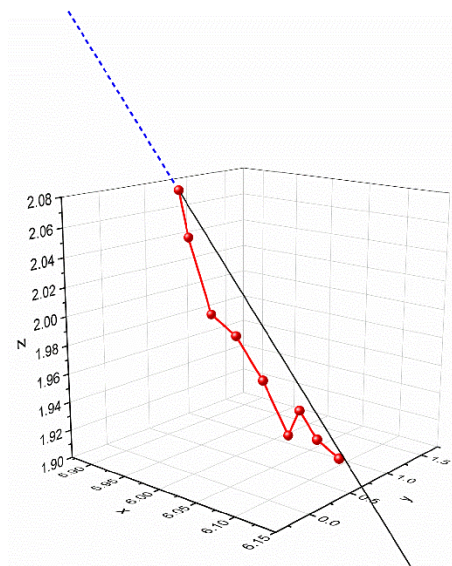


Figure 4.3. Potential displacement trajectories (dotted blue and solid black) from the molecular centroid at the lowest pressure (red dot) to the two possible endpoints (not shown). The correct sense of displacement (solid black line) is determined by agreement with the endpoint dataset.

Once the correct endpoint is selected, the Cartesian coordinates of the molecular centroid and the corresponding trajectory endpoint are stored in a PDB file for three-dimensional visualisation. In this context, the molecular centroid and the selected model endpoint are represented as unknown atoms. Connectivity is specified between the two unknown atoms, resulting in a straight bond between them being displayed when visualising the file in Mercury (Figure 4.2). The bond serves as a graphical representation of the displacement path of m_n relative to m_0 .

This file is designed to be overlaid on the crystal structure at the lowest pressure studied. This approach follows the strategy used by Bond in the *processPIXEL* program⁵⁹, where energy-vector models are also generated in a PDB format for three-dimensional visualisation in Mercury. To ensure accurate overlay of the molecular displacement models onto the crystal structure, the PDB file includes the unit cell parameters from the lowest-pressure crystal structure. It also includes

a transformation matrix to convert Cartesian coordinates into fractional coordinates within the defined unit cell. Together, the unit cell and transformation matrix ensure that the molecular centroid and model endpoint are correctly positioned within the crystallographic frame, allowing for the molecular displacement in the unit cell to be easily visualised and examined.

Step 9: Visualise the molecular movement.

Finally, the PDB file and CIF corresponding to the lowest pressure point are loaded into Mercury for visualisation. The structures are overlaid using the Multiple Structures option and aligned by adjusting the Packing settings (Figure 4.2).

4.4.2. Application of MolDis

A set of acetanilides was used to test MolDis as they provided a range of polymorphs and changes to packing. The translational displacement of molecules under high pressure in paracetamol Form I (4.0 GPa) and Form II (3.55 GPa), the monoclinic form of 4-methylacetanilide (5.33 GPa), 4-chloroacetanilide (5.55 GPa), and metacetamol (3-hydroxyacetanilide) Form I (6.28 GPa) were studied using this tool. The specific pressure points studied and the calculated displacement magnitudes can be found in the Appendix (Appendix Table B4.1). As pressure is applied, the systems retain the same space group, and their unit cell parameters and volumes show no discontinuities, indicating that no sharp phase transitions or structural changes occur within the pressure ranges studied (Appendix Figure B4.25). The discussion that follows first validates the developed method on known structures by comparing the results obtained with those published (i.e., paracetamol Form I and Form II) and then applies the method to structures for which no high-pressure studies have been reported in the literature to investigate the effects of pressure on their crystal structures (i.e., the monoclinic form of 4-methylacetanilide, 4-chloroacetanilide, metacetamol Form I).

4.4.2.1 Paracetamol Form I and Form II

Under ambient conditions, paracetamol Form I and Form II crystallise in the space groups $P2_1/n$ and $Pbca$, respectively^{60–62}. They both have layered hydrogen-bonded structures in which molecules within each layer link via $N-H\cdots O$ and $O-H\cdots O$ hydrogen bonds (Figure 4.4c-d). Adjacent layers interact only by weak van der Waals and π - π interactions. The hydrogen-bonding topology differs between the polymorphs, giving rise to distinct layer geometries (Figure 4.4a-b). This difference in layer geometry governs their mechanical response to pressure: the corrugated layers in Form I restrict glide, resulting in a more brittle response, whereas the nearly planar, more widely spaced layers in Form II permit interlayer slippage and plastic deformation^{62,63}. Previous high-pressure studies up to 4.5 GPa for paracetamol Form I and 5.5 GPa for paracetamol Form II report that compression in both polymorphs is dominated by the direction normal to the layers⁶³. While Form I shows a pressure-induced flattening of the corrugated layers, Form II compresses more evenly within its layers.

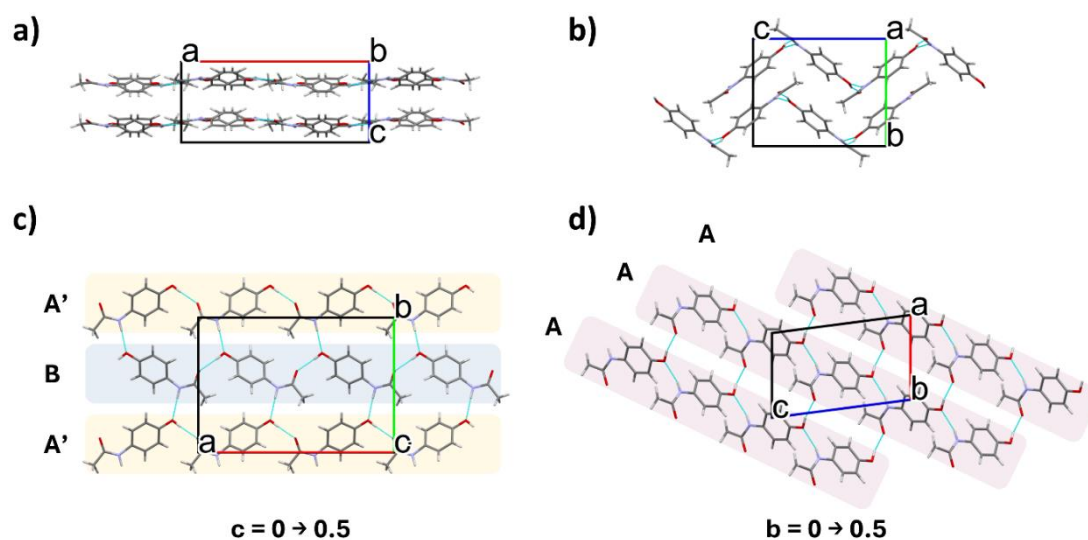


Figure 4.4. Crystallographic structure of paracetamol forms II (a,c) and I (b,d). Frames adapted from Boldyreva et al.⁶³.

Paracetamol Form I was studied using single crystal X-ray diffraction data collected at 0.003 GPa, combined with high-pressure SC-XRD data previously published by Boldyreva *et al.*⁶⁴ and deposited in the Cambridge Structural Database (CSD) under the refcodes HXACAN09-12. Using this combined dataset, MolDis calculated the molecular displacement vectors for paracetamol Form I up to 4.0 GPa. Form I possesses four molecules in the unit cell hence MolDis labels these m_0 to m_3 , where m_0 is the central molecule to which the movement of all other molecules is related (Figure 4.5). For the purposes of this study, m_0 and m_3 are hydrogen bonded in the same hydrogen bonded layer and m_1 and m_2 are in the layer above.

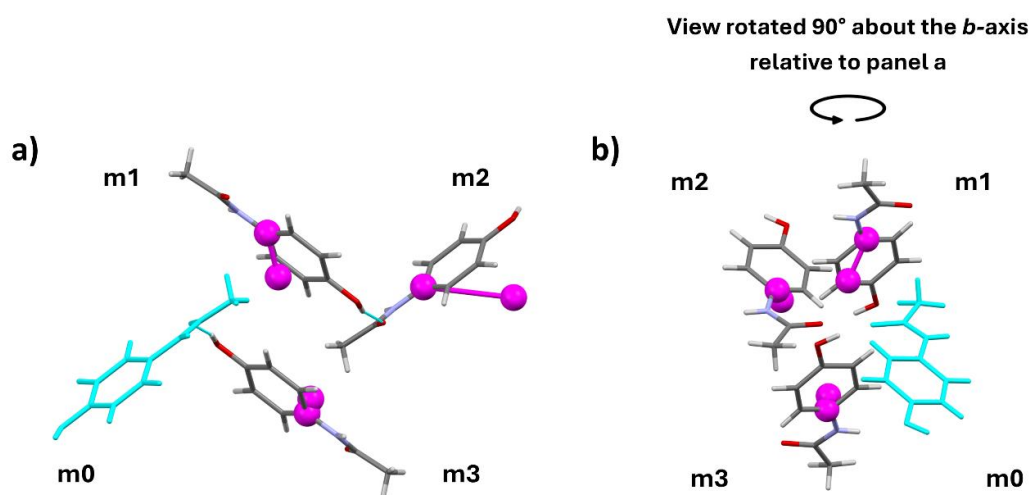


Figure 4.5. Molecular displacement in paracetamol Form I from 0.003 to 4.0 GPa, overlaid on the system's near-ambient structure (0.003 GPa). Displacements are defined relative to the reference molecule (m_0 , cyan molecule) and shown as a pink straight line extending from each molecule's centre of geometry. To facilitate visualisation, displacements are scaled to 10x their calculated magnitude. The calculated magnitudes, in top-to-bottom and left-to-right order from frame a, are: m_2 (0.338), m_1 (0.186), m_3 (0.0549). Frame b is frame a rotated 90° about the z -axis (corresponding to the b -axis in this orientation).

Beginning with the hydrogen-bonded pair, m_3 shows minimal displacement, translating only slightly upwards while preserving its edge-to-edge separation from m_0 (Figure 4.5). This limited movement reflects strong resistance to compression by the hydrogen bond; the interaction energy increases sharply (14.5 kJ mol^{-1}) for a small

reduction in centroid separation (*ca.* 0.03 Å) (Figure 4.6). The steep energy destabilisation shows that this hydrogen-bonded interaction strongly constrains translation under compression.

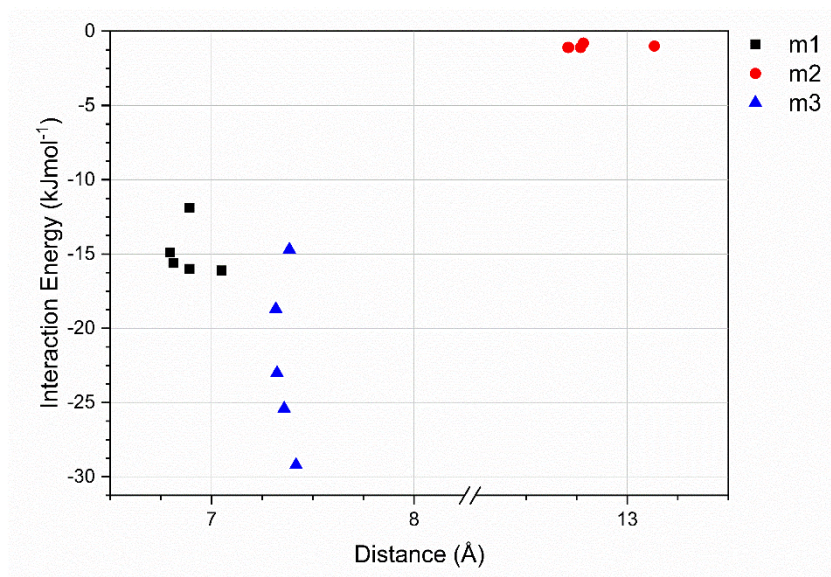


Figure 4.6. Intermolecular interaction energies between the reference molecule (m_0) and molecules in the unit cell of paracetamol Form I, calculated using PIXEL. Molecular labelling follows that shown in Figure 4.5. Total interaction energy is plotted against centroid-centroid distance, with changes occurring as a function of pressure. Symmetry operations for individual interactions: m_0 - m_1 ($0.5-x$, $-0.5+y$, $-0.5-z$), m_0 - m_2 ($1-x$, $1-y$, $-1-z$), m_0 - m_3 ($0.5+x$, $1.5-y$, $-0.5+z$).

Turning to the neighbouring chain, m_1 shifts sideways towards m_2 while translating downwards towards m_0 's chain (Figure 4.5). At lower pressures (0.003 to 2 GPa), this movement occurs with minimal resistance (Figure 4.6). The interaction energy increases only by 0.5 kJ mol⁻¹ despite a reduction in centroid separation of *ca.* 0.24 Å. As the molecules approach more closely (below 6.811 Å, from 2 to 4 GPa), the interaction destabilises rapidly, with the interaction energy increasing sharply by 3.7 kJmol⁻¹ over a small reduction in separation of 0.08 Å. At higher pressures (3 to 4 GPa), the centroid separation increases by *ca.* 0.10 Å despite continued compression, indicating that repulsion limits further approach.

m_2 moves away from m_0 (Figure 4.5) and into a pocket of void space (Figure 4.7), undergoing the largest displacement in the structure. The translation incurs little energetic penalty; the m_0 - m_2 interaction energy is weak and changes only minimally over the pressure range studied (-0.8 kJ mol^{-1} at 0.003 GPa to -1 kJ mol^{-1} at 4 GPa) (Figure 4.6). The separation between the molecules' centroids decreases slightly by *ca.* $0.08 \text{ \AA}$ (starting at *ca.* $12.78 \text{ \AA}$, 0.003 to 2 GPa) before increasing by *ca.* $0.43 \text{ \AA}$ at higher pressures (2 to 4 GPa), indicating that the molecules do not continue to approach under compression but instead reorganise to occupy available free space.

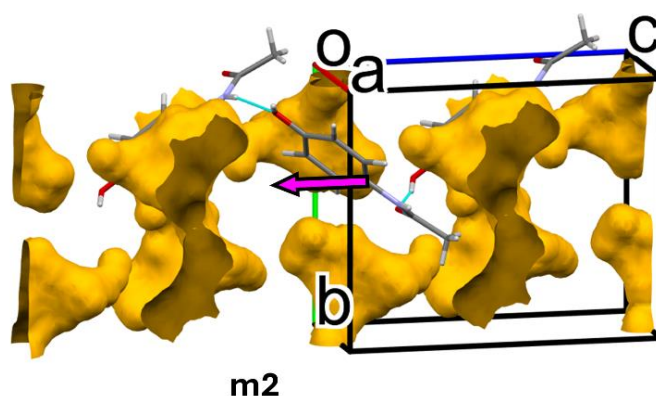


Figure 4.7. Molecular displacement vector from Figure 4.5 overlaid on a depiction of the voids in paracetamol Form I at near ambient pressure (0.003 GPa). The void volume is 13.7% of the unit cell volume using a $0.6 \text{ \AA}$ probe radius and a grid spacing of $0.2 \text{ \AA}$.

Overall, compression in paracetamol Form I is accommodated through limited and localised molecular displacements. The largest displacements from m_2 and m_1 contribute to an apparent gradual flattening of the corrugated molecular layer, consistent with the pressure-induced chain flattening previously reported by Boldyreva *et al.*⁶⁴.

A visualisation of molecular movement in paracetamol Form II up to 3.55 GPa shows that, unlike Form I, Form II accommodates compression normal to the

molecular layers through large displacements that bring layers closer together (Figure 4.8). Despite Form II being studied to a lower pressure than Form I (4.0 GPa), the calculated displacement magnitudes are substantially larger (a maximum of 0.54 Å compared to *ca.* 0.34 Å for Form I), indicating that compression is accommodated by larger displacements throughout the structure rather than by subtle, local translations seen in Form I. Form II possesses eight molecules in the unit cell; MolDis labels these m_0 to m_7 where m_0 is the central molecule to which the movement of all other molecules is related (Figure 4.8). For the purposes of this study, m_5 and m_7 are hydrogen-bonded to m_0 and, together with m_2 , sit on the same layer as m_0 . m_1 , m_3 , m_4 and m_6 lie in the layer below.

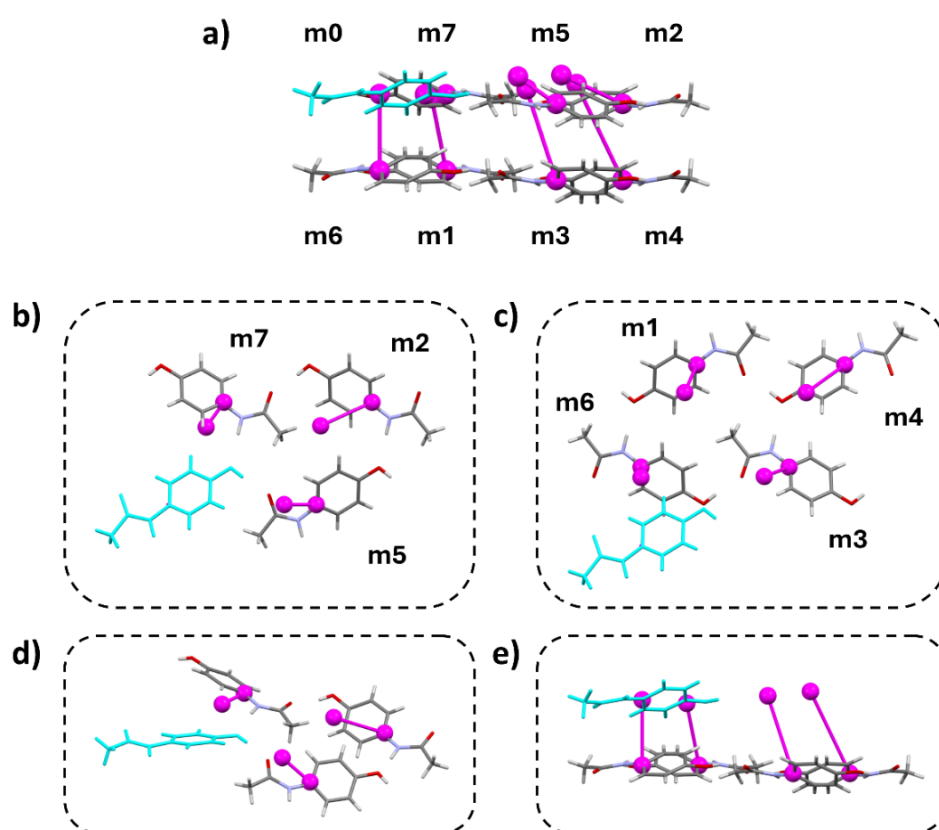


Figure 4.8. Molecular displacement in paracetamol Form II from 0.003 to 3.55 GPa, overlaid on the system's near-ambient structure (0.003 GPa). Displacements are defined relative to the reference molecule (m_0 , cyan molecule) and shown as a pink straight line extending from each molecule's centre of geometry. To facilitate visualisation, displacements are scaled to 10x their calculated magnitude. The calculated magnitudes, in left-to-right order for frames b-c, are: m_7 (0.176), m_2 (0.355), m_5 (0.228), m_6 (0.376), m_1 (0.393), m_3 (0.466), m_4 (0.54).

MolDis shows that all molecules in the unit cell of paracetamol Form II move towards the reference molecule (m_0) upon compression (Figure 4.8). It quantifies a larger displacement magnitude for the molecules on the layer adjacent to m_0 , bringing layers closer along the c -axis. When the compression of the unit cell is accounted for, the displacement trajectory of the molecules in the adjacent layer is roughly normal to the layer's plane, consistent with the behaviour reported in the literature⁶³. The molecules in the adjacent layer also shift to a similar height along the c -axis, preserving the layer's flat geometry.

Void analysis shows that the ambient structure of paracetamol Form II contains pockets of void space in the ab plane repeated periodically along the c -axis (Figure 4.9). MolDis indicates that the displacement trajectories of the molecules on the layer adjacent to m_0 (i.e., m_6 , m_1 , m_3 and m_4) are not directed into discrete void pockets; instead, their translation along the c -axis results in an overall reduction of these unoccupied regions.

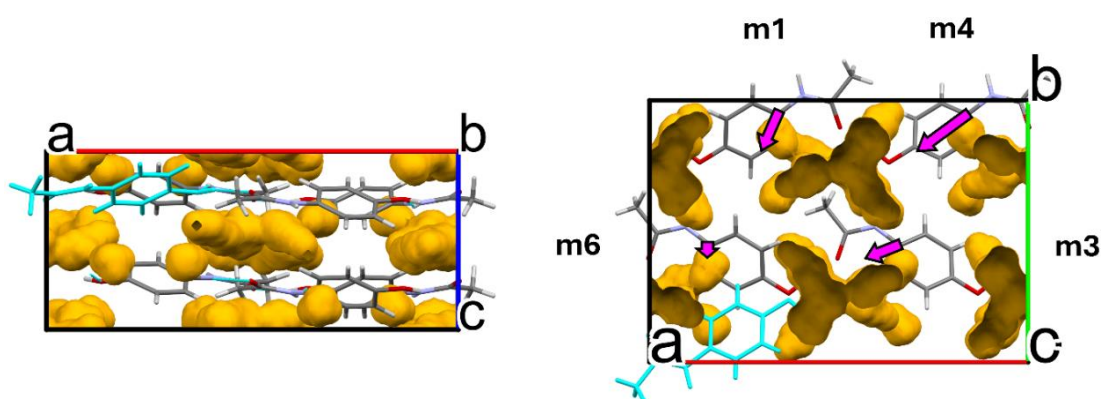


Figure 4.9. Molecular displacement vector from Figure 4.8 overlaid on a depiction of the voids in paracetamol Form II at near ambient pressure (0.003 GPa). The void volume is 11.0% of the unit cell volume using a 0.6 Å probe radius and a grid spacing of 0.2 Å.

The pockets of void space are associated with the (002) slip plane, a slip plane with a flat topology, a separation of 1.082 Å and no bridging hydrogen bonds⁵⁴ (Appendix Table B4.3, Appendix Figure B4.31). Slip planes are crystallographic planes characterised by weak intermolecular interactions between adjacent molecular layers that, under applied shear stress (i.e., stress parallel to the plane), allow these layers to glide relative to one another⁶⁵. The greater plasticity and improved tableting performance of paracetamol Form II compared to Form I are attributed to the presence of this slip plane^{62,66}. Under hydrostatic compression, the (002) slip plane appears to facilitate molecular translation normal to the plane, enabling the progressive filling of the unoccupied space between molecular layers.

The individual contacts are considered from the closest bonded interactions outward, beginning with the molecules in m_0 's own layer. m_7 , m_5 and m_2 undergo small displacements to approach a similar height along the c -axis as m_0 (Figure 4.8). Molecules m_5 and m_7 are both hydrogen-bonded to m_0 and, despite differences in interaction energy (-35.4 kJmol^{-1} for m_0 - m_5 , -27 kJmol^{-1} for m_0 - m_7 at 0.003 GPa), they exhibit a similar compression and steep destabilisation upon compression (Figure 4.10). Over the pressure range studied, the m_0 - m_5 interaction destabilises by 5.2 kJmol^{-1} as the centroid separation decreases by *ca.* 0.23 Å, while the m_0 - m_7 interaction destabilises by 5.4 kJmol^{-1} with a reduction in centroid separation of *ca.* 0.17 Å. In contrast, the weak interaction between m_0 and molecule m_2 remains unchanged upon compression, from -0.2 kJmol^{-1} at 0.003 GPa to -0.2 kJmol^{-1} at 3.55 GPa, indicating that this displacement is governed by steric repulsion from neighbouring molecules rather than by an increase in repulsion from compressing the m_0 - m_2 interaction.

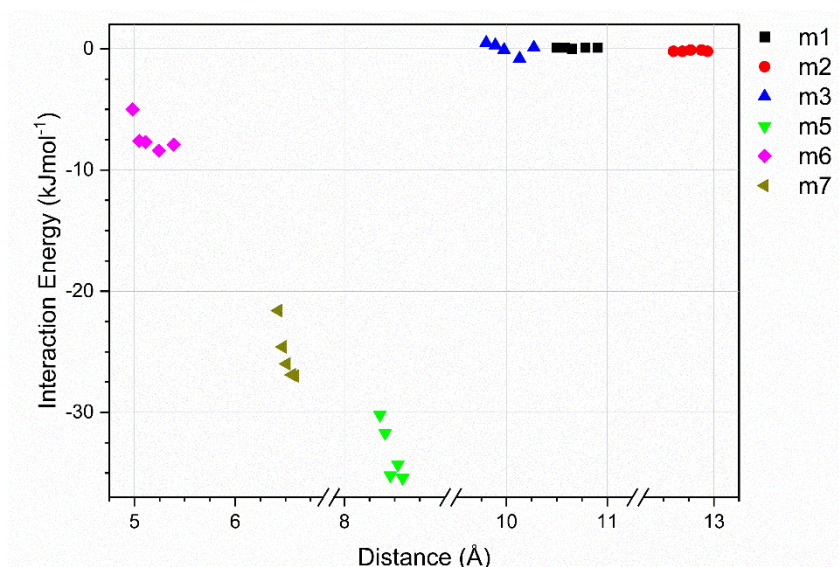


Figure 4.10. Intermolecular interaction energies between the reference molecule (m_0) and molecules in the unit cell of paracetamol Form II, calculated using PIXEL. Molecular labelling follows that shown in Figure 4.8. Total interaction energy is plotted against centroid-centroid distance, with changes occurring as a function of pressure. Symmetry operations for individual interactions: m_0 - m_1 ($1.5-x$, $1-y$, $0.5+z$), m_0 - m_2 ($1-x$, $0.5+y$, $0.5-z$), m_0 - m_3 ($-0.5+x$, $0.5-y$, $1-z$), m_0 - m_4 ($1-x$, $1-y$, $1-z$), m_0 - m_5 ($-0.5+x$, y , $0.5-z$), m_0 - m_6 (x , $0.5-y$, $0.5+z$), m_0 - m_7 ($1.5-x$, $0.5+y$, z).

Turning to the adjacent layer, m_6 , the closest molecule to m_0 by centroid separation, translates directly towards m_0 along the c -axis (Figure 4.8). The weak, largely dispersive interaction remains mostly unchanged up to 2.57 GPa, with the interaction energy decreasing by 0.3 kJmol^{-1} as the molecules' centroids approach by 0.34 Å (Figure 4.10). Beyond this point, the interaction begins to destabilise sharply, increasing by 2.6 kJ mol^{-1} as the centroid separation decreases by *ca.* 0.07 Å , likely due to increased steric repulsion from m_0 's molecular layer.

m_1 and m_3 also translate along the c -axis, but they shift within the ab plane towards m_0 (Figure 4.8). The weak m_0 - m_1 interaction remains unchanged under compression, from 0.1 kJmol^{-1} at 0.003 GPa to 0.1 kJmol^{-1} at 3.55 GPa. The weak m_0 - m_3 interaction gently stabilises at lower pressures, from 0.1 kJmol^{-1} at 0.003 GPa to -0.8 kJmol^{-1} at

0.67 GPa, but later gently destabilises until reaching 0.5 kJmol⁻¹ at 3.55 GPa (Figure 4.10). The greater change in m_3 is likely due to its shorter centroid separation from m_0 .

m_4 is furthest from m_0 within the adjacent layer and lies outside the distance range considered for PIXEL calculations. Given its larger centroid separation, the interaction is expected to be weak and unlikely to vary significantly with compression; its displacements are instead influenced by available void space and steric repulsion with neighbouring molecules.

Across molecular layers, MolDis indicates that compression is accommodated by coordinated molecular translations that reduce intermolecular separation while preserving the local molecular arrangement, consistent with the behaviour reported in the literature⁶³ (Figure 4.8). Within each molecular layer, the molecule furthest from m_0 shows the largest displacement magnitude (m_2 in m_0 's layer, m_4 in the adjacent layer). Because displacements are measured relative to m_0 , this is mostly an effect of the unit cell shrinking under pressure rather than a substantial difference in how these molecules respond.

Overall, MolDis reveals that paracetamol Form II accommodates compression through collective molecular displacement towards m_0 , resulting in a predominant reduction in interlayer separation along the *c*-axis while preserving the molecular arrangement within molecular layers. Hydrogen-bonded contacts in paracetamol Form II exhibit a greater compression and less destabilisation (*ca.* 5 kJmol⁻¹, *ca.* 0.2 Å) than in Form I (14.5 kJmol⁻¹, *ca.* 0.03 Å), despite Form II being studied to a lower pressure (3.55 GPa) than Form I (4.0 GPa). This suggests that, although a closer approach of hydrogen-bonded molecules is strongly repulsive, differences in molecular packing allow Form II to accommodate the compression of the hydrogen-bonded interaction

more readily than Form I. The direction and extent of the reduction in interlayer separation are governed by available void space associated with the (002) slip plane and weaker intermolecular interactions in this direction.

The behaviour observed from the visualisations of molecular displacement generated using MolDis is consistent with the behaviour reported in the literature for paracetamol Form I and Form II, thereby validating the method. The directional information provided by the displacement vectors further supports and extends observations previously made by other research groups. With the performance of the method established for paracetamol Form I and Form II, MolDis is next applied to systems that have not previously been studied crystallographically under high pressure, beginning with the monoclinic form of 4-methylacetanilide.

4.4.2.2 Monoclinic form of 4-methylacetanilide

4-Methylacetanilide is a derivative of paracetamol with the substitution of the hydroxyl group with a methyl group. 4-Methylacetanilide is polymorphic, and two anhydrous crystal forms, monoclinic and orthorhombic, have been reported in the literature^{29,67}. In this study, only the monoclinic form is investigated under pressure, as attempts to obtain the orthorhombic form were unsuccessful. The ambient-pressure phase of the monoclinic form of 4-methylacetanilide crystallises in space group $P2_1/c$. Molecules interact through hydrogen bonded chains (N-H $\cdots$ O interactions) extending along the b -axis (Figure 4.11a). Along the hydrogen-bonded chain, the molecules form a shallow corrugated layer. The chains formed by the molecules translated in a -direction show no hydrogen bonding interactions and are stabilised by the interaction of the methyl group of the amide and the aromatic groups. This layer is repeated above by symmetry but displaced along the b -direction (Figure 4.11b-c).

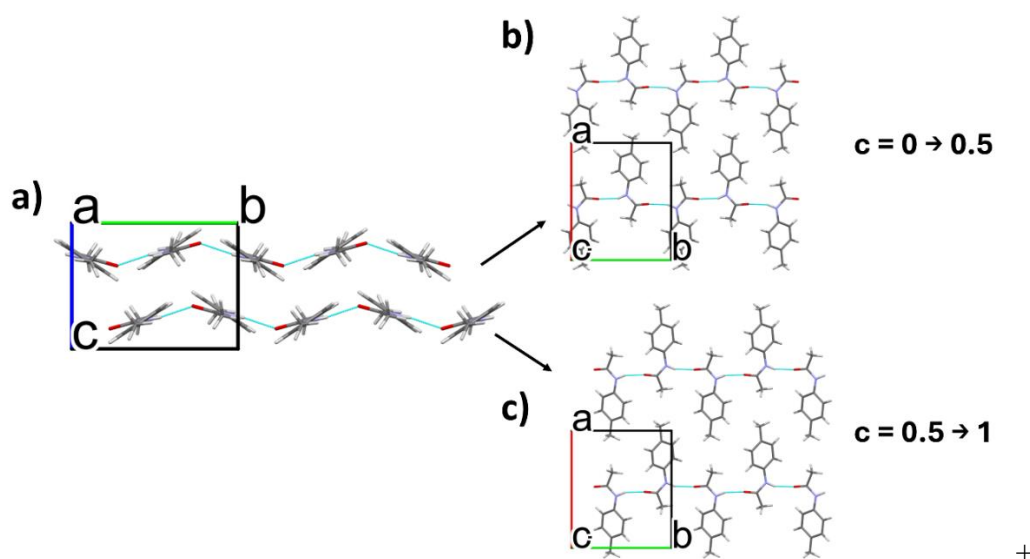


Figure 4.11. Crystallographic structure of the monoclinic form of 4-methylacetanilide.

The monoclinic form of 4-methylacetanilide is studied up to 5.33 GPa. MolDis indicates that the displacement magnitudes are broadly similar throughout the structure (mean 0.43 Å) and comparable to those calculated for paracetamol Form II (mean 0.36 Å). The monoclinic form of 4-methylacetanilide possesses four molecules in the unit cell hence MolDis labels these m_0 to m_3 where m_0 is the central molecule to which the movement of all other molecules is related (Figure 4.12). For the purposes of this study, m_0 and m_1 are hydrogen bonded in the same hydrogen-bonded chain, and m_2 and m_3 are in the chain below.

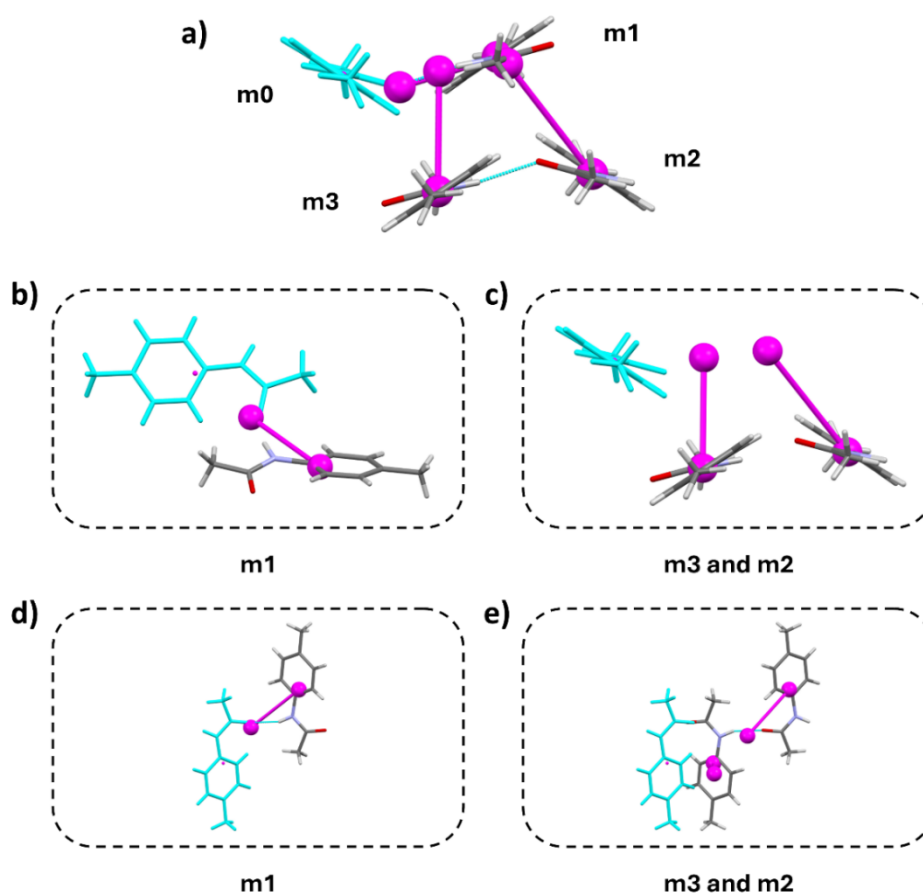


Figure 4.12. Molecular displacement in the monoclinic form of 4-methylacetanilide from 0.06 to 5.33 GPa, overlaid on the system's near-ambient structure (0.06 GPa). Displacements are defined relative to the reference molecule (m_0 , cyan molecule) and shown as a pink straight line extending from each molecule's centre of geometry. To facilitate visualisation, displacements are scaled to 10x their calculated magnitude. The calculated magnitudes, in left-to-right order for frames b-e, are: m_1 (0.423), m_3 (0.376), m_2 (0.48).

Beginning with the hydrogen-bonded pair, m_1 translates towards m_0 by increasing edge-to-edge contact, with minimal change in chain height (Figure 4.12). The predominantly Coulombic interaction between the two molecules destabilises steeply by 11.3 kJmol^{-1} as the centroid separation decreases by *ca.* $0.45 \text{ \AA}$ over the pressure range studied (Figure 4.13), indicating resistance to further approach. This approach contrasts with the limited reduction in centroid separation between hydrogen-bonded contacts in paracetamol Form I (*ca.* $0.03 \text{ \AA}$) and Form II (*ca.* $0.23 \text{ \AA}$, *ca.* $0.17 \text{ \AA}$).

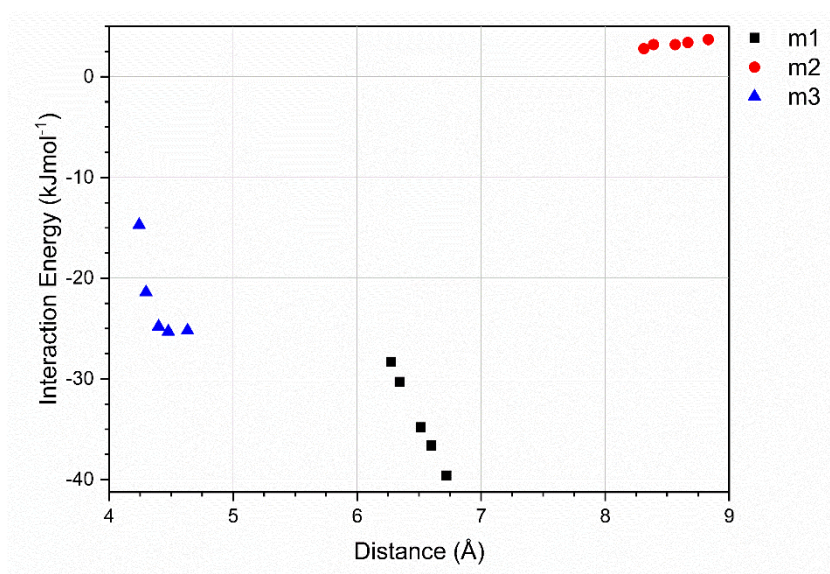


Figure 4.13. Intermolecular interaction energies between the reference molecule (m_0) and molecules in the unit cell of the monoclinic form of 4-methylacetanilide, calculated using PIXEL. Molecular labelling follows that shown in Figure 4.12. Total interaction energy is plotted against centroid-centroid distance, with changes occurring as a function of pressure. Symmetry operations for individual interactions: m_0 - m_1 ($1-x, 0.5+y, 0.5-z$), m_0 - m_2 ($1-x, 1-y, 1-z$), m_0 - m_3 ($x, 0.5-y, 0.5+z$).

Before describing the two molecules in the chain below, it is worth noting the void space between molecular layers (Figure 4.14). Pockets of void space distributed across the *ab* plane are associated with the potential (002) slip plane, a slip plane with a relatively flat topology, a separation of $0.741 \text{ \AA}$ and no bridging hydrogen bonds, identified using the Slip Planes tool in the CSD Particle suite (Appendix Table B4.3,

Appendix Figure B4.31). The interactions between molecular layers (i.e., m_0 - m_3 , m_0 - m_2) are weaker than those within the same layer (i.e., m_0 - m_1) and exhibit predominantly dispersive attractive contributions. As in paracetamol Form II, under hydrostatic compression, this potential slip plane appears to facilitate molecular displacements predominantly normal to the plane, resulting in the progressive filling of unoccupied space between the layers.

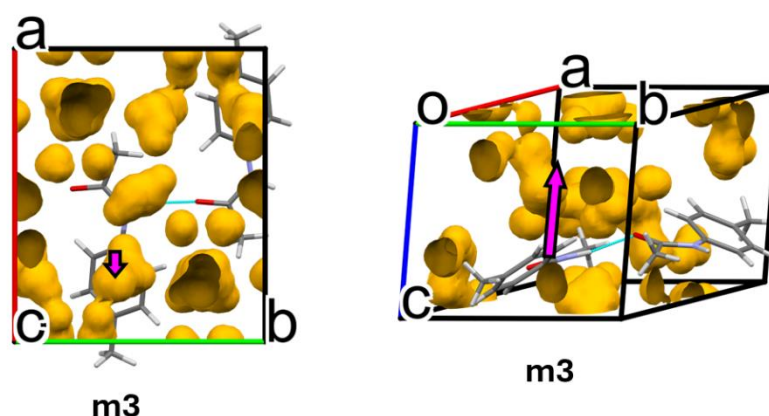


Figure 4.14. Molecular displacement vector from Figure 4.12 overlaid on depictions of the voids in the monoclinic form of 4-methylacetanilide at near ambient pressure (0.06 GPa). The void volume is 9.2% of the unit cell volume using a 0.6 Å probe radius and a grid spacing of 0.2 Å.

m_3 displaces upward along the c -axis and into one of the pockets of void space above itself, overlapping with m_1 (Figure 4.12). The predominantly dispersive interaction between m_0 and m_3 remains largely unchanged as the molecules approach by *ca.* 0.23 Å to a centroid-centroid distance of 4.399 Å, with an interaction energy increase of 0.4 kJmol⁻¹ (Figure 4.13). Beyond this point, the interaction destabilises sharply, increasing by 10.1 kJmol⁻¹ as the centroid separation decreases by *ca.* 0.16 Å between 1.51 and 5.33 GPa, limiting further approach. This suggests that initial displacement makes use of available void space, while further approach is progressively limited by increasing repulsive contributions. This behaviour is similar

to that observed in the dispersive interaction between m_0 and m_1 in paracetamol Form I.

m_2 translates towards m_0 's chain while simultaneously moving closer to m_3 , increasing edge-to-edge contact (Figure 4.12). It also adopts a similar height along the c -axis to m_3 , indicating that the shallow corrugation of the chains is largely preserved upon compression. This is consistent with the modest decrease in the (002) layer root-mean-square deviation (RMSD) from 0.527 at 0.06 GPa to 0.479 at 5.33 GPa. The larger lateral translation of m_2 compared to m_3 corresponds to a shortening of the $N-H\cdots O$ hydrogen bond (Figure 4.15). The weak interaction between m_0 and m_2 stabilises only slightly as the centroid separation decreases, from 3.7 kJmol⁻¹ at 0.003 GPa to 2.8 kJmol⁻¹ at 5.33 GPa (Figure 4.13), suggesting that displacement at higher pressures is limited by steric repulsion from neighbouring m_3 rather than by repulsive contributions from compressing the interaction with m_0 .

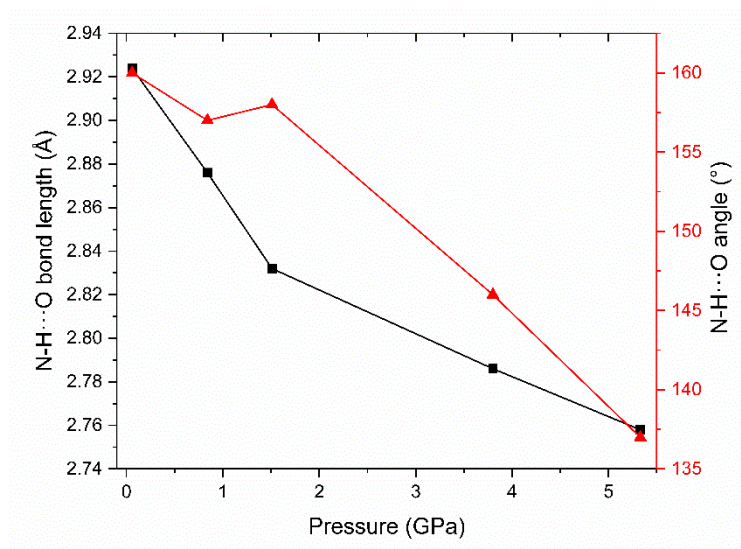


Figure 4.15. Pressure dependence of $N-H\cdots O$ bond length and angle in the monoclinic form of 4-methylacetanilide.

Overall, MolDis indicates that compression in the monoclinic form of 4-methylacetanilide is primarily accommodated through reduced interchain separation along the *c*-axis and increased edge-to-edge contact within the chains, resulting in a closer packing while preserving chain topology. The direction and extent of displacement are governed by the available void space associated with the potential (002) slip plane and repulsive intermolecular interactions. This behaviour aligns with the system's principal axes of strain (Appendix Table B4.2), reflecting a compression that reduces interlayer separation while preserving the chains' topology.

4.4.2.3 4-Chloroacetanilide

4-Chloroacetanilide is a derivative of paracetamol in which the hydroxyl group is replaced with a chlorine atom. It crystallises in the space group $Pna2_1$ at ambient pressure^{27,68}. Like the monoclinic form of 4-methylacetanilide, molecules in 4-chloroacetanilide pack in chains, with hydrogen-bonded chains extending along the a -axis formed by intermolecular $N-H\cdots O$ interactions. It resembles Form I of paracetamol more closely, as hydrogen bonding occurs between molecules whose molecular planes lie at 66° from each other. (Figure 4.16a).

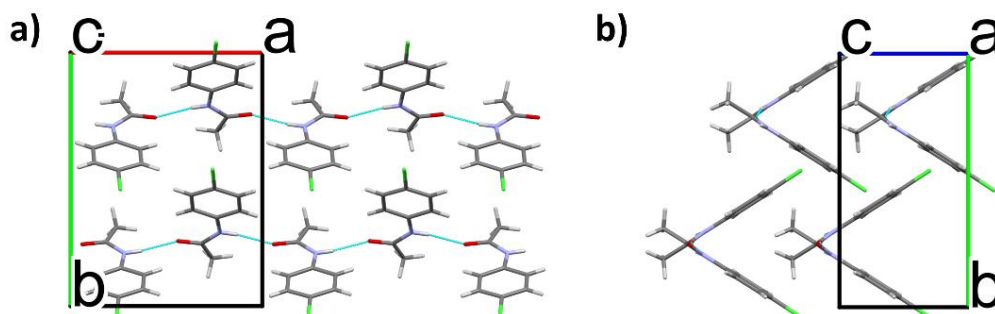


Figure 4.16. Crystallographic structure of 4-chloroacetanilide.

4-chloroacetanilide has been studied up to 5.55 GPa. The molecular displacement magnitudes in 4-chloroacetanilide are the largest in the set of systems studied. At a maximum magnitude of 0.943 Å, they are *ca.* 75-97% larger than those in paracetamol Form II (0.54 Å) and the monoclinic form of 4-methylacetanilide (0.48 Å). This occurs despite the interaction energies being commensurate across the three systems (Figure 4.10, Figure 4.13, Figure 4.20). 4-Chloroacetanilide possesses four molecules in the unit cell hence MolDis labels these m_0 to m_3 where m_0 is the central molecule to which the movement of all other molecules is related (Figure 4.17). For the purposes of this

study, m_0 and m_2 are hydrogen-bonded in the same hydrogen-bonded chain, and m_1 and m_3 are in the chain below.

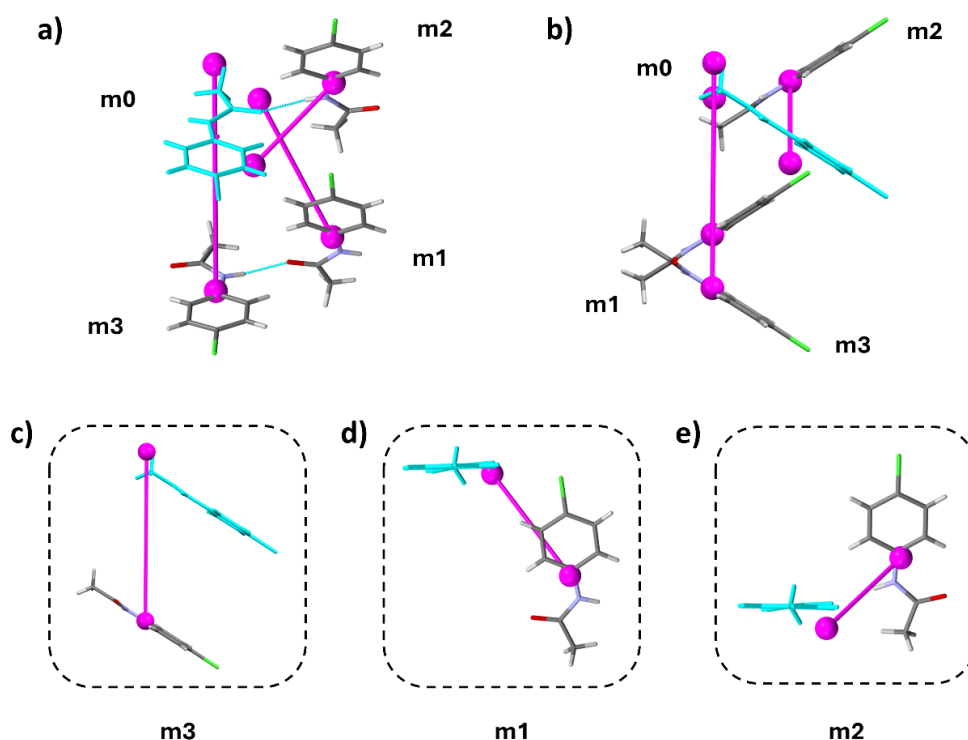


Figure 4.17. Molecular displacement in 4-chloroacetanilide from 0.08 to 5.55 GPa, overlaid on the system's near-ambient structure (0.08 GPa). Displacements are defined relative to the reference molecule (m_0 , cyan molecule) and shown as a pink straight line extending from each molecule's centre of geometry. To facilitate visualisation, displacements are scaled to 10x their calculated magnitude. The calculated magnitudes, in left-to-right order from frames b-d, are: m_2 (0.479), m_3 (0.943), m_1 (0.648).

Void analysis shows the ambient structure of 4-chloroacetanilide contains pockets of void space arranged in pseudo-2D sheets in the bc plane, periodically repeated along the a -axis, into which molecules can translate under compression (Figure 4.18).

Beginning with the hydrogen-bonded contact, m_2 translates laterally towards m_0 (Figure 4.17) and into a pocket of void space (Figure 4.18). This translation reduces the projected chain width along the bc plane and increases edge-to-edge contact between hydrogen-bonded molecules. At the same time, the tilt angle across the bc plane between the molecular planes of neighbouring molecules on the same chain

decreases, from 66° at 0.08 GPa to 57° at 5.55 GPa. The N-H $\cdots$ O bond length shortens with increasing pressure. The bond angle becomes more linear as molecules approach 1.07 GPa, then reverts towards its ambient geometry (Figure 4.19). The predominantly Coulombic interaction between m_2 and m_0 destabilises substantially by 12.4 kJmol^{-1} , reflecting the energetic penalty associated with shortening hydrogen-bonded contacts (Figure 4.20). Despite this destabilisation, the molecular centroids still approach by $0.55 \text{ \AA}$, a magnitude comparable to that of the hydrogen-bonded contact in the monoclinic form of 4-methylacetanilide (*ca.* $0.45 \text{ \AA}$).

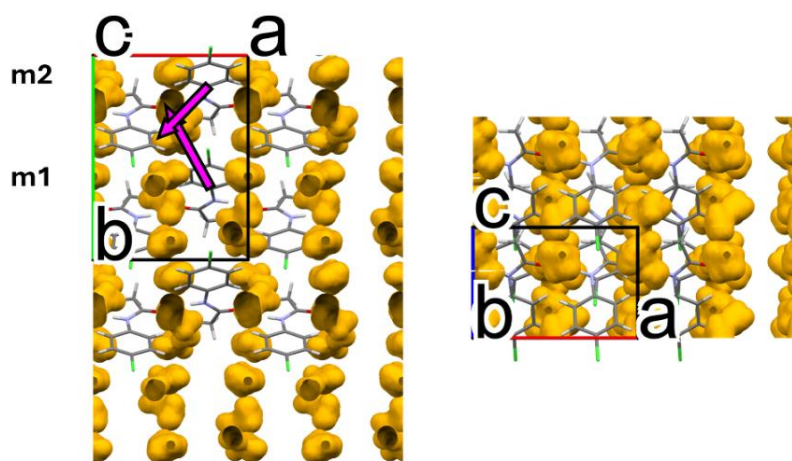


Figure 4.18. Depiction of the voids in 4-chloroacetanilide at near ambient pressure (0.08 GPa). The void volume is 12.0% of the unit cell volume using a $0.6 \text{ \AA}$ probe radius and a grid spacing of $0.2 \text{ \AA}$. (Left) Void space distribution with molecular displacement vectors from Figure 4.17 overlaid. (Right) Void space distribution only.

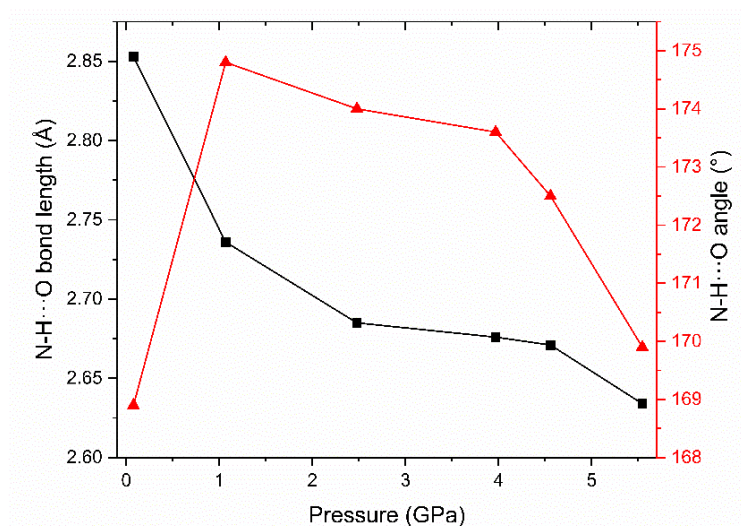


Figure 4.19. Pressure dependence of N-H...O bond length and angle in 4-chloroacetanilide.

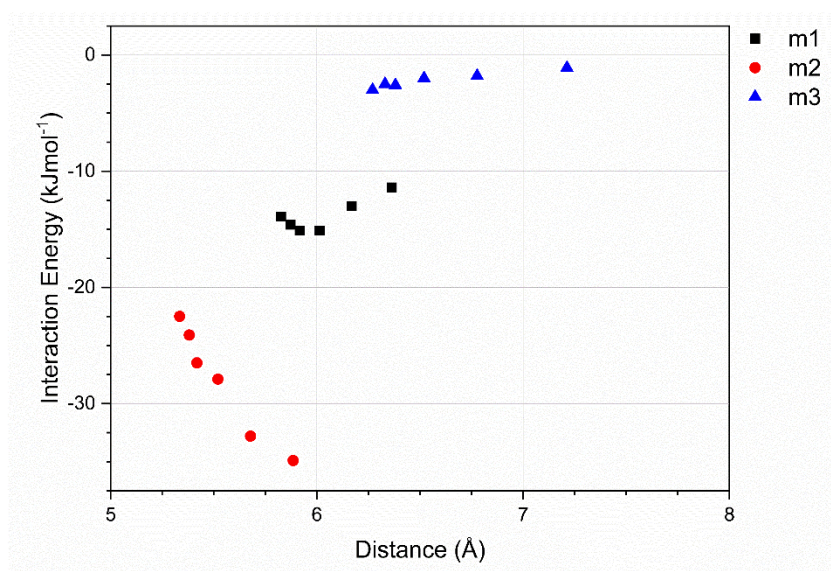


Figure 4.20. Intermolecular interaction energies between the reference molecule (m_0) and molecules in the unit cell of 4-chloroacetanilide, calculated using PIXEL. Molecular labelling follows that shown in Figure 4.17. Total interaction energy is plotted against centroid-centroid distance, with changes occurring as a function of pressure. Symmetry operations for individual interactions: m_0 - m_1 ($1-x, 1-y, 0.5+z$), m_0 - m_2 ($0.5+x, 0.5-y, z$), m_0 - m_3 ($0.5-x, 0.5+y, 0.5+z$).

Turning to the neighbouring chain, m_3 , the molecule stacked face-to-face above m_0 , translates towards m_0 along the b -axis offsetting the stacking (Figure 4.17). Their centroid separation decreases by *ca.* 0.94 Å. The weak interaction between m_0 and m_3 stabilises slightly as the molecules approach, from -1.1 kJmol⁻¹ at 0.08 GPa to -3 kJmol⁻¹ at 5.55 GPa, indicating that the displacement is energetically favourable (Figure 4.20).

m_1 , on the same chain as m_3 , translates upward along the b -axis to reduce the separation between neighbouring chains, while simultaneously displacing laterally towards m_0 and into a pocket of void space, adopting a lower b -axis height than m_3 (Figure 4.17). This displacement reduces the projected width of the chain along the bc plane while increasing edge-to-edge contact between the two molecules. The predominantly dispersive interaction between m_0 and m_1 remains energetically favourable during the initial compression, stabilising by 3.7 kJmol⁻¹ as the centroid-centroid distance decreases by *ca.* 0.45 Å until reaching 5.917 Å (Figure 4.20). Beyond this point, further approach is limited, with the interaction energy destabilising by 1.2 kJmol⁻¹ as the centroid separation decreases by only 0.09 Å due to the increasing steric repulsion between m_1 and m_3 .

The Slip Planes tool in the CSD Particle suite identified two potential slip planes, (200) and (020); whether a contact is described below as within a slab or across slabs is defined relative to whichever plane is under discussion. The pockets of void space in 4-chloroacetanilide are associated with the potential (200) slip plane, a slip plane with a relatively flat topology, a separation of 0.624 Å and bridging hydrogen bonds (Appendix Table B4.3, Appendix Figure B4.31). Relative to this plane, bridging m_0 - m_2 hydrogen bonds cross adjacent slabs and their interaction is stronger than the m_0 -

m_1 and m_0 - m_3 contacts which lie within a slab, and becomes increasingly repulsive under compression. Glide across the (200) plane is therefore unlikely, and this slip plane is not expected to facilitate plastic deformation.

The (020) slip plane does not have bridging hydrogen bonds and has the widest interlayer separation in 4-chloroacetanilide (Appendix Table B4.3, Appendix Figure B4.31). Relative to this plane, the m_0 - m_1 and m_0 - m_3 contacts cross the adjacent slabs and are weaker than the m_0 - m_2 hydrogen bonds that lie within a slab; nonetheless, the (020) slip plane has a corrugated topology and an interdigitation of 0.992 Å that would likely hinder plastic deformation of the structure under applied shear stress.

Overall, MolDis indicates that 4-chloroacetanilide accommodates compression through molecular displacements that make use of available void space and increase edge-to-edge molecular contact, bringing neighbouring chains closer together along the *b*-axis and contributing to chain flattening as the chain length along the *a*-axis weakly compresses. This response resembles that of the monoclinic form of 4-methylacetanilide, where compression promotes the approach of neighbouring chains and increased molecular contact within chains, and is consistent with the system's principal axes of strain (Appendix Table B4.2). In this system, the impact of crystallographic symmetry on the molecular displacements is particularly clear. The directions of displacement are strongly shaped by the symmetry operations relating each molecule to the reference. This is worth keeping in mind, as the main goal of this analysis is to uncover the individual molecular response that is missed by conventional analyses based on changes to the unit cell.

4.4.2.4 Metacetamol Form I

The final structure studied is metacetamol Form I. Metacetamol has been reported to crystallise in two anhydrous polymorphs, Form I³⁰ and Form II⁶⁹. Only Form I is investigated under pressure, as Form II is reported to have a weak stability and single crystals exhibit poor crystallinity for diffraction studies.⁶⁹

Metacetamol Form I crystallises in the space group $Pna2_1$ under ambient conditions and forms a three-dimensional hydrogen-bonded network. Within the bc plane, molecules link by intermolecular O-H $\cdots$ O interactions to form pseudo-chains (Figure 4.21a), which connect to each other by N-H $\cdots$ O interactions to form dimers and C(6) motifs along the ab plane, giving rise to pseudo-layers that extend along the c -direction. Similar to 4-chloroacetanilide, adjacent molecules within these chains tilt relative to one another, adopting an angle of *ca.* 62° at 0.003 GPa (Figure 4.21b). Pseudo-chains stack parallel along the c -axis and alternate in orientation along the a -axis, sitting at an angle of *ca.* 69° relative to each other at 0.003 GPa (Figure 4.21c).

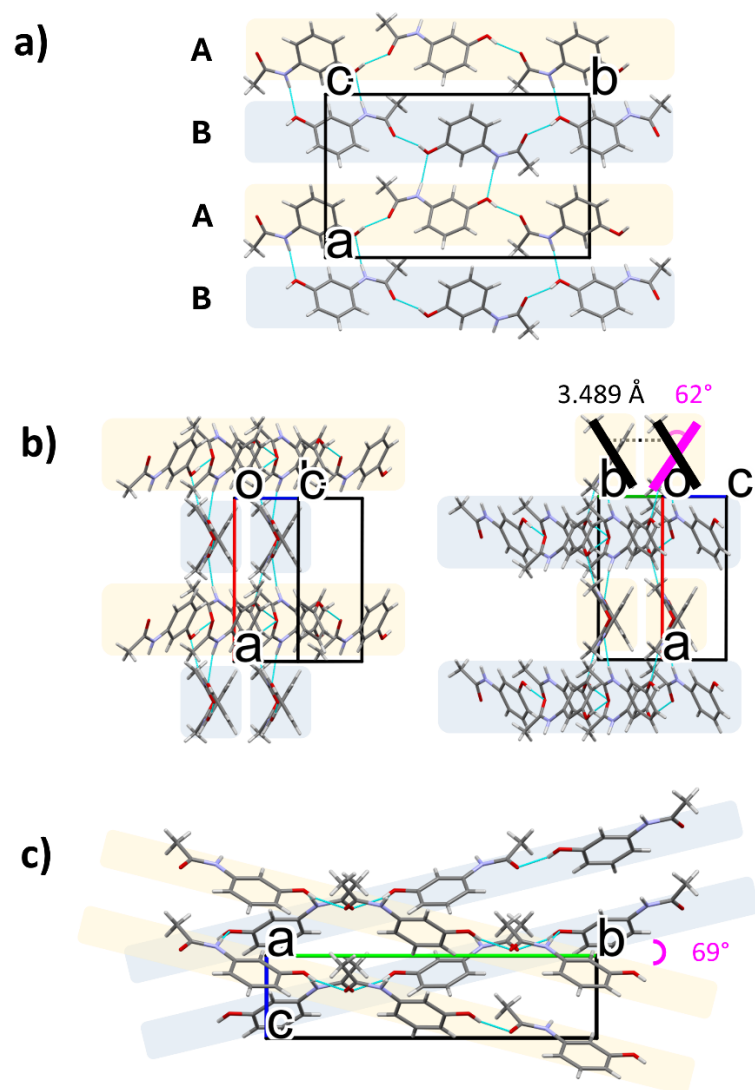


Figure 4.21. Crystallographic structure of metacetamol Form I.

The displacement vectors calculated by MolDis show that the magnitude of molecular displacement in metacetamol Form I is the second smallest in the series despite being studied to the highest pressure in the series (6.28 GPa). The maximum magnitude of 0.428 Å is smaller than that in paracetamol Form II (0.54 Å), the monoclinic form of 4-methylacetanilide (0.48 Å) and 4-chloroacetanilide (0.943 Å), and only slightly larger than in paracetamol Form I (0.338 Å). Metacetamol Form I possesses four molecules in the unit cell hence MolDis labels these m_0 to m_3 where m_0

is the central molecule to which the movement of all other molecules is related (Figure 4.22). For the purposes of this study, m_0 and m_1 are hydrogen bonded, and m_2 and m_3 neighbour them in the three-dimensional hydrogen-bonded network.

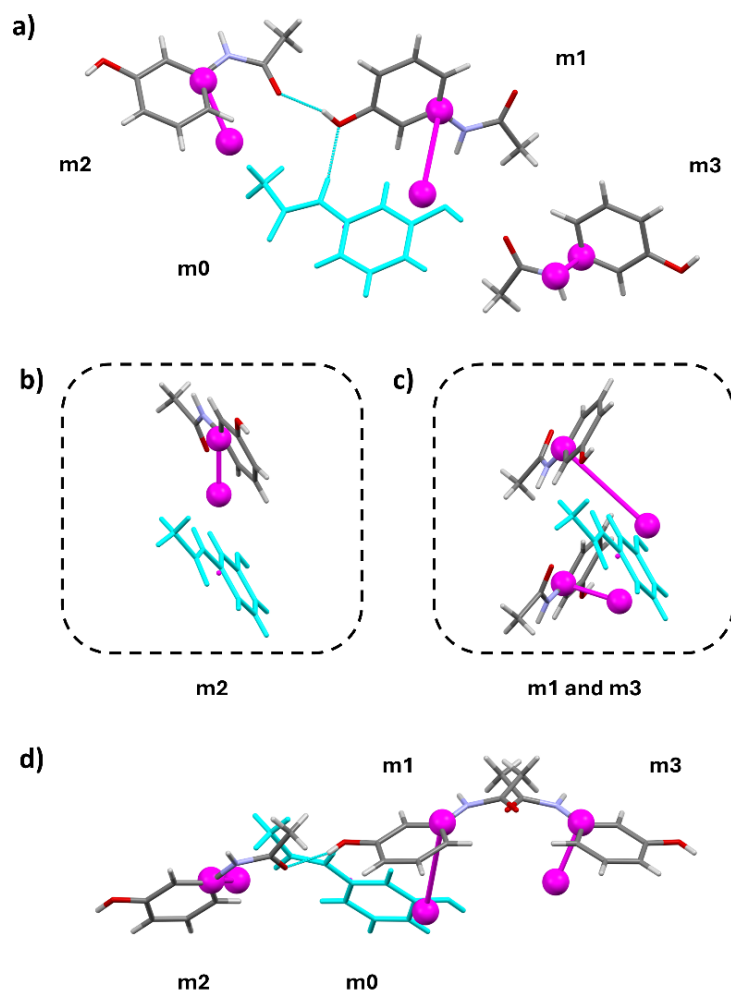


Figure 4.22. Molecular displacement in metacetamol Form I from 0.003 to 6.28 GPa, overlaid on the system's near-ambient structure (0.003 GPa). Displacements are defined relative to the reference molecule (m_0 , cyan molecule) and shown as a pink straight line extending from each molecule's centre of geometry. To facilitate visualisation, displacements are scaled to 10x their calculated magnitude. The calculated magnitudes, in left-to-right order for frames b-c, are: m_2 (0.238), m_1 (0.428), m_3 (0.242).

Beginning with the hydrogen-bonded contact, m_1 translates into the pockets of void space beneath it (Figure 4.23) as it approaches m_0 (Figure 4.22). This displacement brings neighbouring pseudo-chains closer along the a -axis. m_1 undergoes the largest reduction in centroid separation relative to m_0 in the system (*ca.* 0.42 Å) despite the

steep destabilisation of the interaction with increasing pressure and rising repulsion (8.1 kJmol^{-1} increase) (Figure 4.24). This indicates that, although the interaction becomes energetically unfavourable, molecular displacement and compression of the hydrogen bond are not fully inhibited. Similar behaviour occurs in the monoclinic form of 4-methylacetanilide and 4-chloroacetanilide, where hydrogen-bonded molecules continue to approach despite increasing repulsion (centroids approach by *ca.* $0.45 \text{ \AA}$ and $0.55 \text{ \AA}$, respectively).

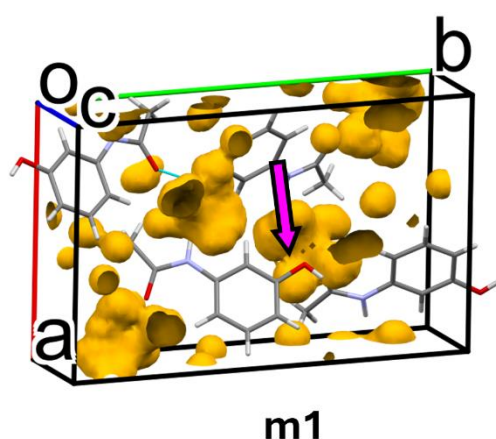


Figure 4.23. Molecular displacement vector from Figure 4.22 overlaid on a depiction of the voids in metacetamol Form I at near ambient pressure (0.003 GPa). The void volume is 10.0% of the unit cell volume using a $0.6 \text{ \AA}$ probe radius and a grid spacing of $0.2 \text{ \AA}$.

m_2 undergoes a displacement downwards towards the reference molecule (m_0), bringing neighbouring pseudo-chains closer together (Figure 4.22). The interaction between m_0 and m_2 gently stabilises by 2.2 kJmol^{-1} as the centroid-centroid distance is reduced by *ca.* $0.21 \text{ \AA}$ (Figure 4.24), indicating that it is energetically favourable for the molecules to approach one another. This displacement is coupled with a decrease in the angle between the mean planes of the two molecules, from *ca.* 26° at 0.003 GPa to *ca.* 8° at 6.28 GPa , effectively aligning them and contributing to the reduced separation.

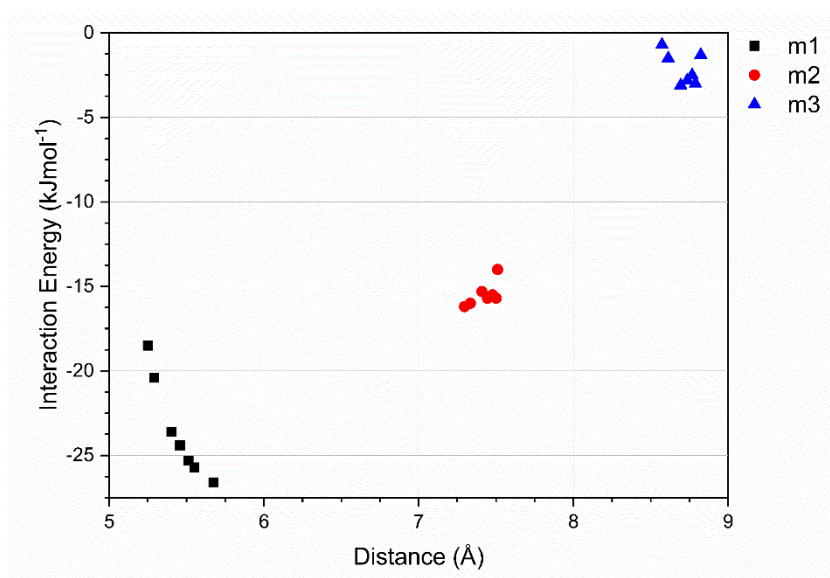


Figure 4.24. Intermolecular interaction energies between the reference molecule (m_0) and molecules in the unit cell of metacetamol Form I, calculated using PIXEL. Molecular labelling follows that shown in Figure 4.22. Total interaction energy is plotted against centroid-centroid distance, with changes occurring as a function of pressure. Symmetry operations for individual interactions: m_0 - m_1 ($1-x, 1-y, -0.5+z$), m_0 - m_2 ($-0.5+x, 0.5-y, z$), m_0 - m_3 ($1.5-x, 0.5+y, -0.5+z$).

m_3 translates along the c -axis towards m_0 with little lateral approach (Figure 4.22). The weak interaction strengthens from -1.3 kJmol^{-1} at 0.003 GPa to -3.1 kJmol^{-1} at 2.61 GPa as the centroid separation decreases to 8.694 Å (Figure 4.24). Beyond this point, further approach becomes unfavourable, with the interaction destabilising by 2.4 kJmol^{-1} up to 6.28 GPa. At higher pressures, the displacement magnitude is likely limited by steric repulsion from a symmetry-equivalent molecule in a neighbouring unit cell along the c -axis rather than by repulsive contributions from compressing the m_0 - m_3 interaction. This displacement reduces the separation between pseudo-molecular layers along the c -axis (Figure 4.22b), with the spacing between parallel-stacked layers decreasing by *ca.* 0.56 Å over the pressure range studied.

Overall, MolDis shows that metacetamol Form I accommodates compression through multi-directional molecular displacements of small magnitudes that collectively reduce the overall unit cell volume while largely preserving the ambient pressure packing arrangement. Unlike the other systems studied, the behaviour resolved does not directly reflect the system's principal axes of strain (Appendix Table B4.2).

Principal strain analysis indicates that pressure is accommodated primarily by bringing pseudo-layers closer through a predominant compression of the *c*-axis, with a substantially softer compression of the *a*-axis and then the *b*-axis. MolDis, by contrast, shows comparable molecular displacements along the *a*- and *c*-axes, with similar reductions in distance between the reference molecule and molecules in pseudo-chains and pseudo-layers. The two are not in conflict but describe different things. Principal strain analysis captures the net deformation of the unit cell, whereas MolDis vectors report the relative displacement of individual molecules. This is where MolDis adds value, as it resolves molecular-level behaviour that a unit cell-level analysis averages over.

4.4.3. Overview of the application of MolDis

The case studies presented in this Chapter collectively demonstrate how MolDis captures different structural responses under pressure. For paracetamol Form I and Form II, the only systems with previously reported high-pressure structural data, the behaviour identified from molecular displacements is consistent with that reported in the literature, thereby validating the method. For the monoclinic form of 4-methylacetanilide, 4-chloroacetanilide, and metacetamol Form I, for which no equivalent datasets have been published, the analysis provides the first experimental description of their responses to pressure.

From the visualisation of molecular displacement vectors, it is possible to identify how molecules approach one another and relate these local translations to interaction energies and structural features such as void space or potential slip planes. The method therefore provides mechanistic insight beyond that obtained from conventional crystallographic analyses, such as strain analysis, by capturing the direction and relative magnitude of molecular displacement.

From these observations, four general trends emerge across the acetanilide systems studied:

- Molecules in all systems, with the exception of paracetamol Form I, generally move towards the reference molecule. These displacements compress the structures, bringing molecules closer together while largely preserving the ambient packing arrangements across one-dimensional chains, two-dimensional layers, and three-dimensional hydrogen-bonded networks.
- Corrugated molecular chains and layers tend to flatten under pressure, as seen for paracetamol Form I and 4-chloroacetanilide.

- All systems contain hydrogen-bonded interactions that become steeply destabilised under compression. However, hydrogen-bonded molecules approach each other more in the monoclinic form of 4-methylacetanilide (*ca.* 0.45 Å, 5.33 GPa), 4-chloroacetanilide (0.55 Å, 5.55 GPa), and metacetamol Form I (*ca.* 0.42 Å, 6.28 GPa) than in paracetamol Form I (*ca.* 0.03 Å, 4.0 GPa) and Form II (*ca.* 0.23 Å and *ca.* 0.17 Å, 3.55 GPa). The corrugated geometry of the molecular layers in Form I appears to inhibit molecular translation in comparison to the planar layers of Form II.
- Widely spaced potential slip planes with no bridging hydrogen bonds across slabs, identified using the Slip Planes tool in the CSD Particle suite and associated with void space in the structure, appear to facilitate directional molecular displacement (i.e., normal to the plane). This is seen for paracetamol Form II, for which the (002) slip plane is linked to enhanced plasticity and improved tableting performance, and the monoclinic form of 4-methylacetanilide.

MolDis provides molecular-level information on how pressure is accommodated within all the crystalline structures studied. In doing so, it distinguished systems whose molecular displacements follow a single dominant trend (paracetamol Form II and the monoclinic form of 4-methylacetanilide) from those whose displacements are more complex (paracetamol Form I, 4-chloroacetanilide and metacetamol Form I) (Appendix Figures B24-B28). Approaches that could characterise multi-directional trajectories more fully are outlined in Section 4.4.4.

4.4.4. Limitations of MolDis

Although the method developed in this Chapter offers an intuitive way to visualise molecular displacements under pressure, it also has several limitations.

Firstly, experimental uncertainty must be considered, although its impact is small. This includes systematic errors from single-crystal X-ray diffraction data collection and the limited reciprocal-space coverage inherent to the DAC geometry. These uncertainties manifest as standard uncertainties on the refined atomic positions, which propagate to the centroid positions used in the molecular displacement analysis and the calculated lines of best fit that describe molecular displacement. However, the molecular displacements interpreted in this Chapter exceed the associated centroid uncertainty. The equivalent isotropic atomic displacement parameters (U_{eq}) for atoms in the crystallographic data studied lie between *ca.* 0.02 and 0.07 Å², corresponding to root-mean-square (rms) positional uncertainties of 0.14 to 0.26 Å (i.e., $\sqrt{U_{eq}}$). Because each molecular centroid is defined by 11 non-hydrogen atoms, the uncertainty in a centroid position is reduced by averaging and is therefore only *ca.* 0.04 to 0.08 Å (i.e., $0.14/\sqrt{11}$ to $0.26/\sqrt{11}$). Among all the molecular displacement magnitudes, this centroid uncertainty exceeds only the displacement of m₃ in paracetamol Form I (0.0549 Å). Additional uncertainty arises from the determination of pressure in DAC experiments, which is accurate to within *ca.* 0.05 GPa and represents a small relative uncertainty across the pressure ranges studied.

Secondly, limitations arise from the type of data used. The molecular displacement analysis presented in this Chapter is based on long-range, symmetry-averaged atomic coordinates obtained from X-ray diffraction, meaning local distortions or symmetry-

forbidden displacements cannot be resolved. Pair distribution function (PDF) analysis from total scattering measurements could be used to resolve the local structure⁷⁰.

Thirdly, the method itself has limitations, particularly in representing molecular positions and displacements. Since the position of molecules in space is defined by their centre of geometry, a more thorough analysis would be needed for larger, more flexible molecules to ensure that changes in displacement are not due to conformational change. However, this limitation is not significant for the structure of the acetanilide molecules studied in this Chapter.

Molecular displacement is represented as a single straight vector. Although this representation captures the overall direction of displacement, it does not trace the molecule's path of movement when the direction of displacement changes substantially across the pressure range, as seen in some of the systems studied. In metacetamol Form I, for example, the displacement trajectories of molecules m_1 and m_3 show a pronounced change in direction, approaching a *ca.* 90° turn after 3.80 GPa (Appendix Figure B4.30a,c). Similar non-linear trajectories are observed in paracetamol Form I (directional change after 2.0 GPa for m_1 , m_2 and m_3 ; Appendix Figure B4.26) and in 4-chloroacetanilide (directional change after 1.07 GPa for m_1 and m_3 ; Appendix Figure B4.29a,c).

A related limitation arises from the use of principal component analysis (PCA). In this work, PCA is used to identify the direction of maximum variance to determine a line of best fit through the displacement endpoints, rather than as a statistical tool to decompose and interpret the displacement behaviour. This approach assumes that molecular displacement can be meaningfully approximated by a single dominant direction. When the displacement direction remains approximately constant with

increasing pressure, as seen in paracetamol Form II (Appendix Figure B4.27) and the monoclinic form of 4-methylacetanilide (Appendix Figure B4.28), the first principal component provides a meaningful descriptor of the molecular behaviour. However, for systems with multi-directional displacement behaviour, such as metacetamol Form I, paracetamol Form I and 4-chloroacetanilide, a single PCA-derived best-fit direction averages over multiple displacement regimes, so it captures the overall direction of translation but underrepresents the multi-directional path.

Additional PCA-based analysis, such as examination of the scree plots, would identify the number of statistically significant principal components required to describe the displacement behaviour, providing a quantitative measure of its complexity. Examination of the corresponding loadings would reveal which Cartesian displacement components (i.e., x, y, z vector coordinates) contribute most strongly to each principal component. These analyses lie beyond the scope of this Chapter but represent an extension for future work. They would help establish when a single PCA-derived best-fit direction is adequate and when additional components are required and would help rationalise observed non-linear displacement behaviour.

Finally, interpretative limitations should be acknowledged. PCA is sensitive to the largest sources of variance, meaning that a single large positional change at a given pressure point can disproportionately influence the dominant trend and the resulting direction of molecular displacement. More generally, the output from MolDis is primarily qualitative; it is useful for identifying and visualising molecular translations not captured by other analyses, but robust interpretation requires considering these displacements alongside complementary quantitative analyses, such as interaction energy calculations, and structural descriptors like void space and slip planes.

4.5. Conclusions and Future Work

This Chapter establishes that pressure-induced changes in organic molecular crystals can be examined directly by considering how individual molecules move relative to a reference molecule. It introduces a method and an accompanying tool, MolDis, that enables visualisation of molecular displacement and interpretation of pressure-induced responses that are not readily accessible through conventional crystallographic analyses.

While conventional analyses, such as the principal axes of strain, successfully describe the overall structural response of the unit cell to pressure, they do not directly capture how molecules approach one another. The visualisations generated in this Chapter address this gap by making directional molecular displacement explicit and showing the contributions of individual translations. Molecular displacement analysis is not intended as a standalone analysis; rather, when combined with interaction energy calculations and structural descriptors, such as void space and slip planes, it enhances the interpretation of compression behaviour.

Across the systems studied, compression is accommodated through limited displacement and chain flattening in paracetamol Form I, predominant interlayer translation in paracetamol Form II, and intra- and interchain compression in the monoclinic form of 4-methylacetanilide, interchain translations with chain flattening in 4-chloroacetanilide, and small-magnitude multidirectional displacements in metacetamol Form I. Individual molecular displacement trajectories are governed by structural characteristics such as packing motifs, intermolecular interactions and available void space. For systems with previously reported high-pressure data, molecular displacement visualisations reproduce known compression behaviour,

validating the method's reliability. For systems lacking such data, they provide the first reported experimental description of their structural response under pressure.

Although the method is predominantly qualitative and relies on simplifying assumptions on how displacement is represented, these limitations do not prevent it from resolving the structural trends discussed in this Chapter. Rather, they define clear opportunities for future development. A natural next step is to follow the additional PCA-based analysis detailed in Section 4.4.4 to better capture and rationalise non-linear displacement behaviour, and introduce a quantitative measure of how well the displacement vectors represent molecular movement. In addition, extending the method to track changes in molecular orientation would provide additional information on molecular rearrangements under pressure, enabling the identification of rotations occurring alongside or in lieu of translation.

Further insight could be gained by combining the molecular displacement analysis with complementary approaches not explored in this Chapter, such as terahertz spectroscopy⁷¹ and low-frequency Raman spectroscopy⁷², both of which access local dynamic information not captured by structures determined by X-ray diffraction⁷³. Building on the potential role of slip planes identified in this Chapter, testing additional systems in which slippage is known or suspected to play a role in accommodating pressure would help clarify the relationship between local molecular translations under hydrostatic pressure and macroscopic deformation mechanisms that occur under uniaxial compression. It would also be interesting to validate whether the presence of the potential (002) slip plane in the monoclinic form of 4-methylacetanilide enhances plasticity and improves tableting performance, as in paracetamol Form II.

Applied to larger datasets, the method has the potential to reveal broader trends in how different classes of molecular crystals respond to pressure. Repeating this analysis with more structures and extending the approach to molecular crystals with different core structures, multicomponent systems, or more flexible frameworks, such as metal-organic frameworks, would test its general applicability.

Overall, this work demonstrates that molecular displacement visualisation offers a powerful and intuitive means of analysis. By providing direct insight into how molecules redistribute under pressure, the method enhances our ability to understand and interpret pressure-induced structural change in molecular solids.

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Appendix

Table B4.1. Calculated molecular displacement magnitudes under applied pressure of paracetamol Form I (PCM I), paracetamol Form II (PCM II), the monoclinic form of 4-methylacetanilide (4Me M), 4-chloroacetanilide (4Cl), and metacetamol Form I (MCM I).

	Pressure points studied (GPa)	Magnitudes of molecular displacement between min-max pressures (Å) <i>Listed in numerical order (m_0, m_1, m_2, etc.)</i>
PCM I	0.003, 1.0, 2.0, 3.0, 4.0	0, 0.186, 0.338, 0.0549
PCM II	0.003, 0.27, 0.67, 1.11, 1.70, 2.35, 2.57, 2.86, 3.55	0, 0.393, 0.355, 0.466, 0.54, 0.228, 0.376, 0.176
4Me M	0.06, 0.84, 1.51, 3.80, 5.33	0, 0.423, 0.48, 0.376
4Cl	0.08, 1.07, 2.48, 3.97, 4.56, 5.55	0, 0.648, 0.479, 0.943
MCM I	0.003, 0.85, 1.31, 1.95, 2.61, 5.07, 6.28	0, 0.428, 0.238, 0.242

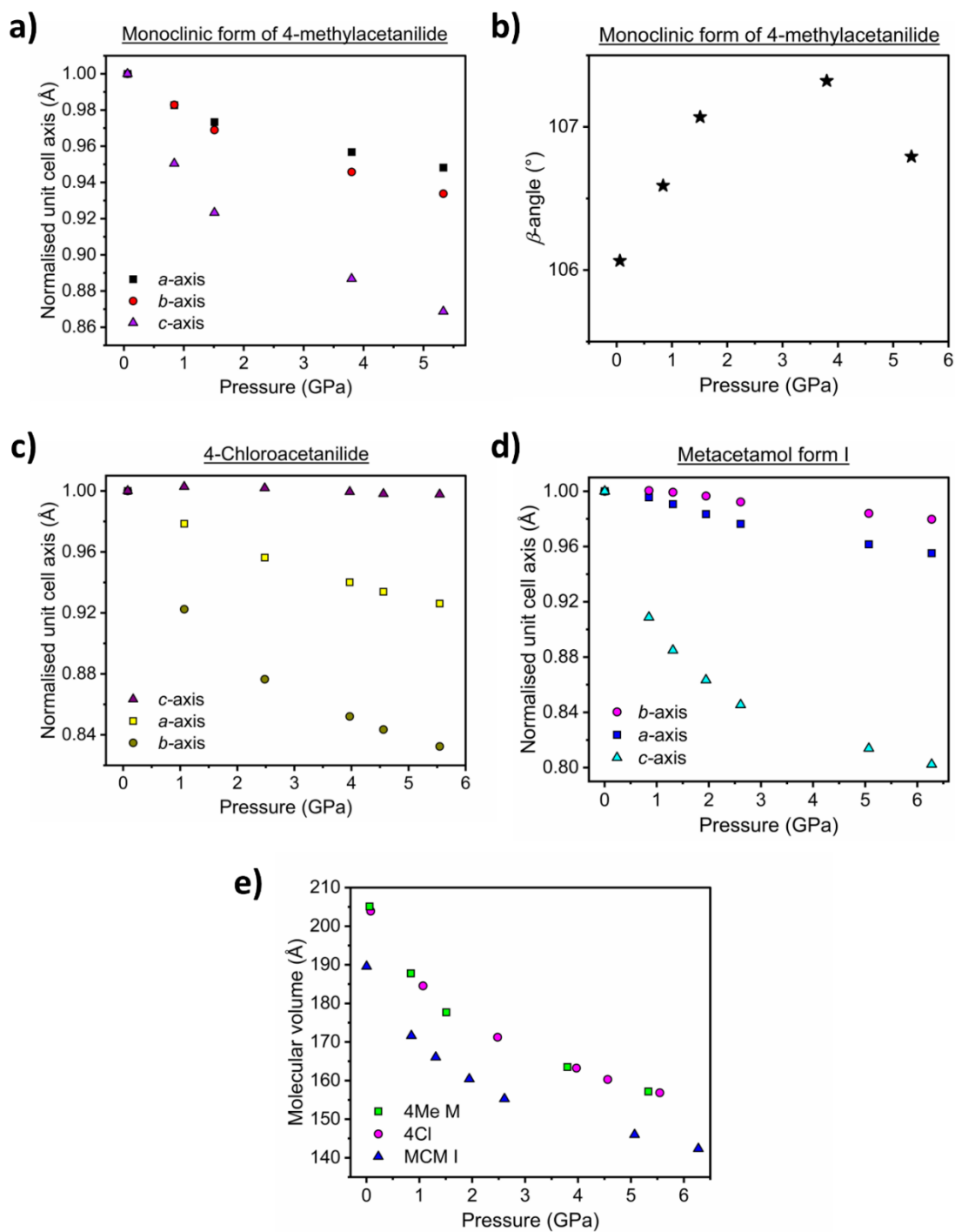


Figure B4.25. a,c-d) Change in the unit cell axes of the monoclinic form of 4-methylacetanilide (4Me M), 4-chloroacetanilide (4Cl) and metacetamol Form I (MCM I) as a function of pressure. b) Change in the β -angle of the monoclinic form of 4-methylacetanilide as a function of pressure. e) Change in the molecular volume of the three systems as a function of pressure.

Table B4.2. Principal axes of strain for the crystal structures of paracetamol Form I (PCM I), paracetamol Form II (PCM II), the monoclinic form of 4-methylacetanilide (4Me M), 4-chloroacetanilide (4Cl), and metacetamol Form I (MCM I). The table shows the direction of compression and the coefficients of compressibility (K).

PRINCIPAL AXES OF STRAIN						
X1			X2		X3	
	Direction	K (TPa ⁻¹)	Direction	K (TPa ⁻¹)	Direction	K (TPa ⁻¹)
PCM I	[0,1,0]	36(6)	[0.9274, 0, 0.3741]	16(4)	[-0.6953, 0, 0.7187]	0(23)
PCM II	[0,0,1]	37(2)	[1,0,0]	7.5(3)	[0,1,0]	7.2(5)
4Me M	[0.1864, 0, 0.9825]	30(11)	[0.9996, 0, -0.0279]	10.8(8)	[0,1,0]	15(2)
4Cl	[0,1,0]	24.8(4)	[1,0,0]	13.5(2)	[0,0,1]	1(1)
MCM I	[0,0,1]	30(2)	[1,0,0]	8(2)	[0,1,0]	3.3(9)

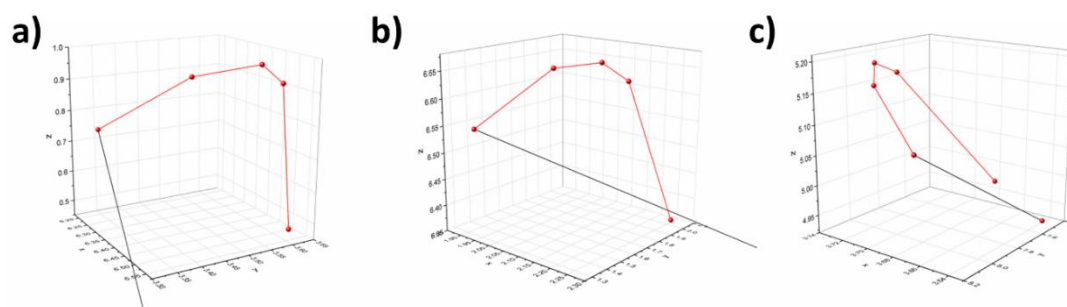


Figure B4.26. Step 8 applied to paracetamol Form I. Three-dimensional representations showing the endpoints (red points) of the translated $m_0 \rightarrow m_n$ vectors for each molecule m_n in the unit cell at all pressure points, together with the determined molecular displacement vector (black line) for each molecule. The molecular displacement vector originates from the endpoint at the lowest pressure studied, with endpoints connected in order of increasing pressure. Panels correspond to (a) m_1 , (b) m_2 , (c) m_3 .

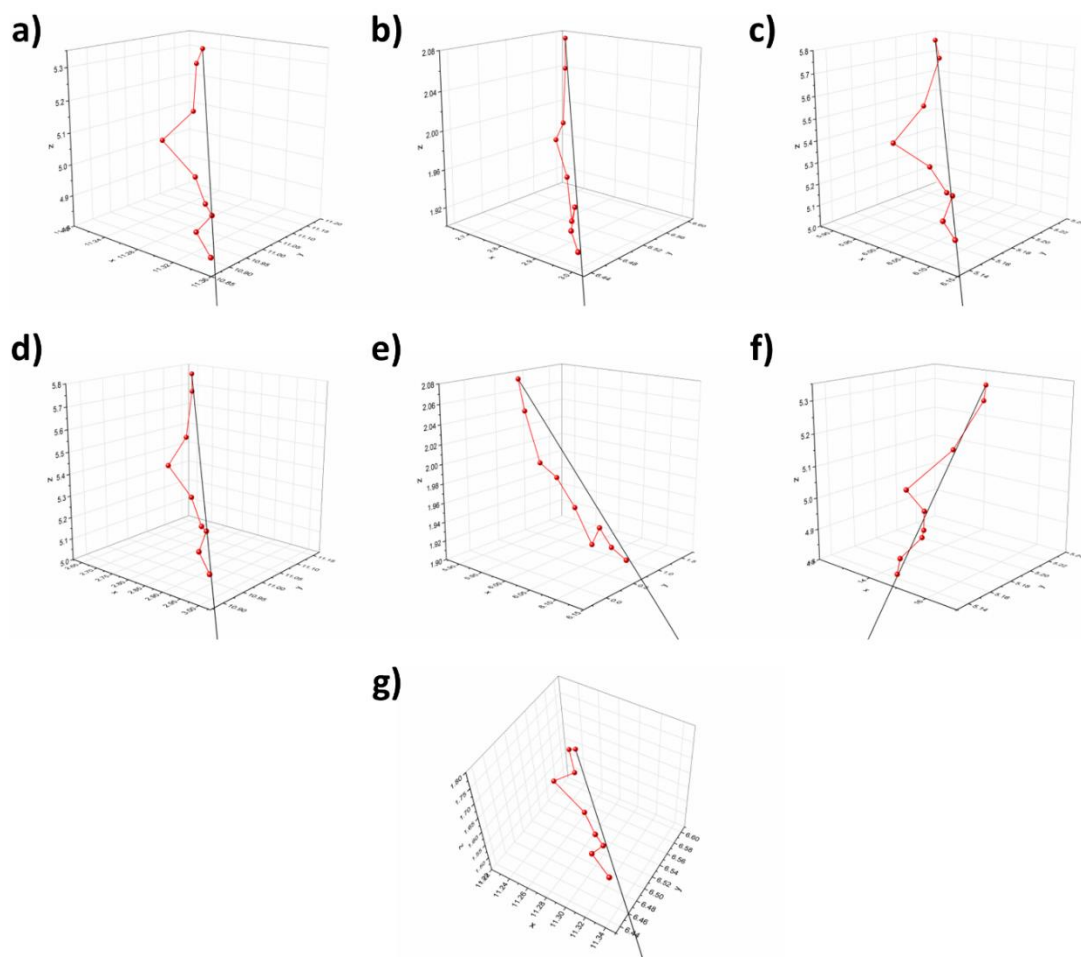


Figure B4.27. Step 8 applied to paracetamol Form II. Three-dimensional representations showing the endpoints (red points) of the translated $m_0 \rightarrow m_n$ vectors for each molecule m_n in the unit cell at all pressure points, together with the determined molecular displacement vector (black line) for each molecule. The molecular displacement vector originates from the endpoint at the lowest pressure studied, with endpoints connected in order of increasing pressure. Panels correspond to (a) m_1 , (b) m_2 , (c) m_3 , (d) m_4 , (e) m_5 , (f) m_6 , (g) m_7 .

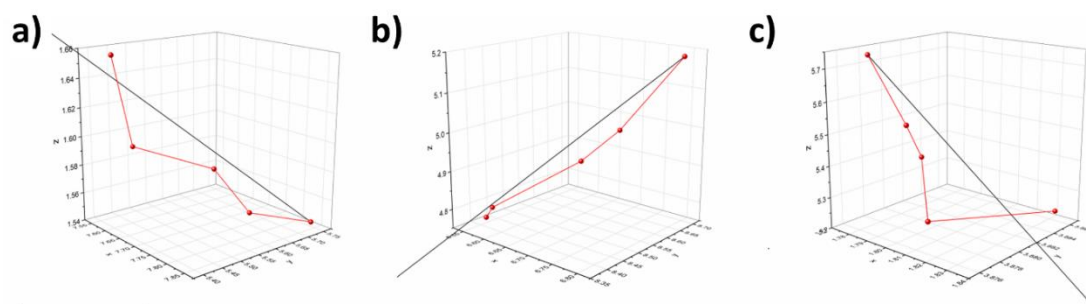


Figure B4.28. Step 8 applied to the monoclinic form of 4-methylacetanilide. Three-dimensional representations showing the endpoints (red points) of the translated $m_0 \rightarrow m_n$ vectors for each molecule m_n in the unit cell at all pressure points, together with the determined molecular displacement vector (black line) for each molecule. The molecular displacement vector originates from the endpoint at the lowest pressure studied, with endpoints connected in order of increasing pressure. Panels correspond to (a) m_1 , (b) m_2 , (c) m_3 .

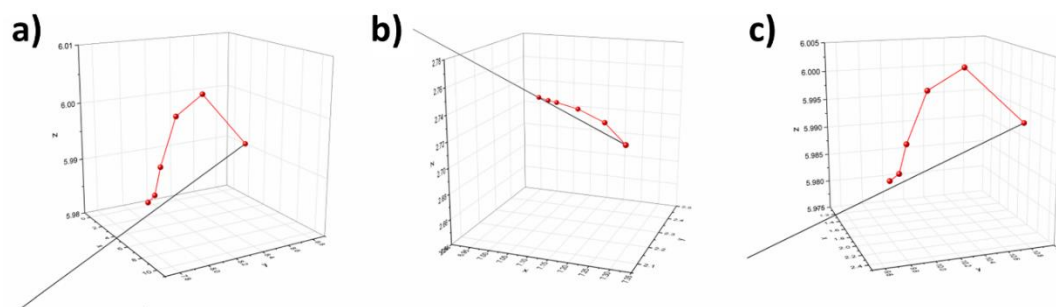


Figure B4.29. Step 8 applied to 4-chloroacetanilide. Three-dimensional representations showing the endpoints (red points) of the translated $m_0 \rightarrow m_n$ vectors for each molecule m_n in the unit cell at all pressure points, together with the determined molecular displacement vector (black line) for each molecule. The molecular displacement vector originates from the endpoint at the lowest pressure studied, with endpoints connected in order of increasing pressure. Panels correspond to (a) m_1 , (b) m_2 , (c) m_3 .

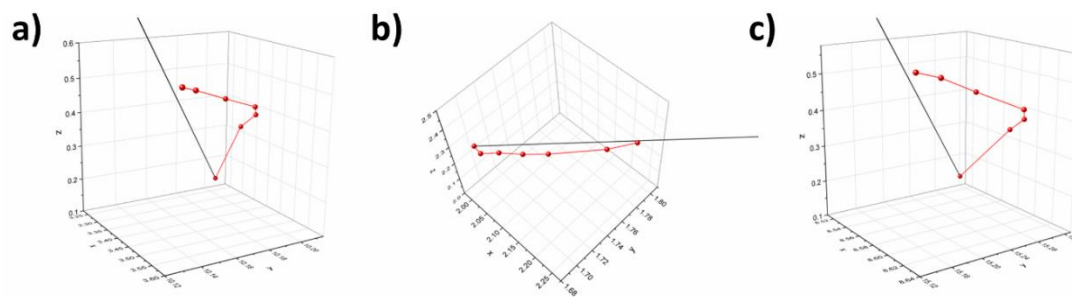


Figure B4.30. Step 8 applied to metacetamol Form I. Three-dimensional representations showing the endpoints (red points) of the translated $m_0 \rightarrow m_n$ vectors for each molecule m_n in the unit cell at all pressure points, together with the determined molecular displacement vector (black line) for each molecule. The molecular displacement vector originates from the endpoint at the lowest pressure studied, with endpoints connected in order of increasing pressure. Panels correspond to (a) m_1 , (b) m_2 , (c) m_3 .

Table B4.3. Potential slip planes identified using the CSD-Particle Slip Plane analysis, showing the crystallographic orientation, slab separation, and the presence or absence of hydrogen-bonding interactions linking neighbouring slabs for each structure studied. Paracetamol Form I (PCM I), paracetamol Form II (PCM II), the monoclinic form of 4-methylacetanilide (4Me M), 4-chloroacetanilide (4Cl), and metacetamol Form I (MCM I).

SLIP PLANES			
	Orientation	Slab separation (Å)	H-bonds bridging
PCM I	No slip planes with positive slab separation		
PCM II	(002)	1.082	No
4Me M	(002)	0.741	No
4Me M	(011)	0.290	Yes
4Me M	(01-1)	0.290	Yes
4Cl	(200)	0.624	Yes
4Cl	(020)	-0.992	No
MCM I	(200)	0.120	Yes

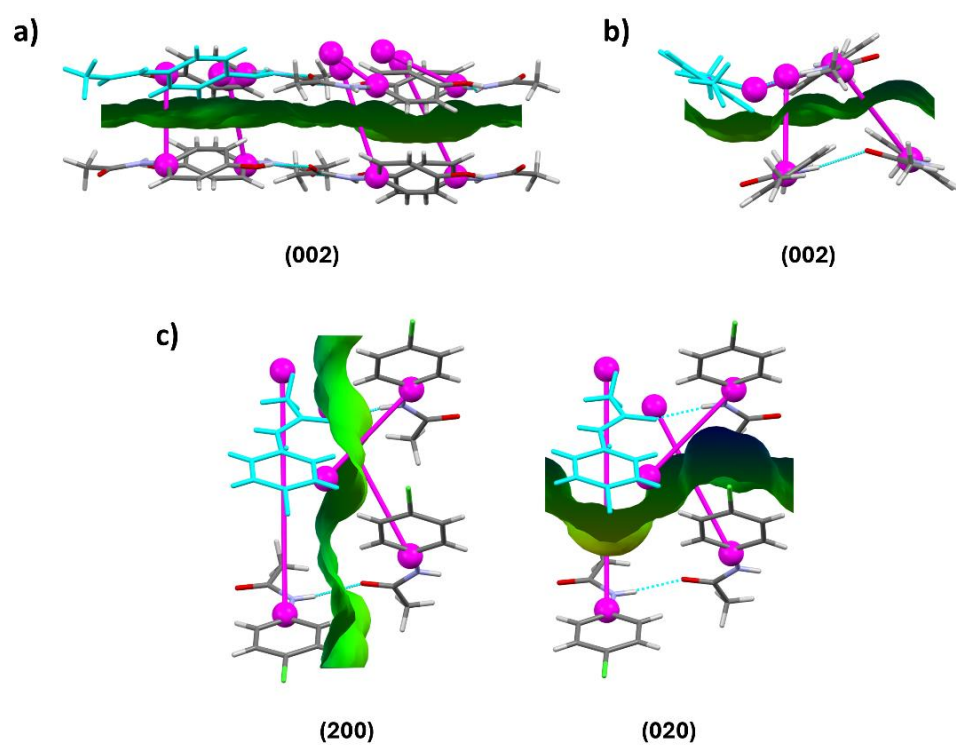


Figure B4.31. Visualisations of the geometry of potential slip planes with positive slab separation and no hydrogen bonding in (a) paracetamol Form II, (b) the monoclinic form of 4-methylacetanilide and (c) 4-chloroacetanilide, with molecular displacements projected onto the slip planes.

Chapter 5 - Spatial and temporal visualisation of polymorphic transformations in pharmaceutical tablets

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5.1. Abstract

X-ray Diffraction Computed Tomography (XRD-CT) represents a cutting-edge method for non-destructive material analysis, offering the unique capability of providing molecular-level information with spatial resolution. In this study, XRD-CT has been applied to investigate pharmaceutically relevant tablets that have been subjected to a range of compression pressures typical in tablet manufacturing. By employing XRD-CT to pharmaceutical tablets, material changes have been revealed without tablet destruction, thereby avoiding potential phase transformations during sample preparation that could lead to errors in the interpretation of the processes that have occurred. Using a pressure-sensitive marker, glycolide, changes within tablet structures induced by compression have been tracked, pinpointing locations where glycolide undergoes pressure-induced transformation. Additionally, a follow-up study with analysis one month later has been conducted, observing an *in situ* hydrolysis reaction of glycolide within the tablets. Through the complementary use of electron diffraction, the structure of the hydrolysis product has been elucidated, further enhancing our understanding of temporal changes in the tablets.

5.2. Introduction

Compression-induced phase transformations in pharmaceutically-relevant organic materials is an area of current interest due to the increasing number of compounds that undergo such changes in pressure ranges relevant to pharmaceutical manufacturing processes¹⁻⁴. These changes have a material impact on the functionality of the compounds giving rise to potential changes in, for example, mechanical properties⁵ and solubility⁶. Changes to the solid form have also been observed at pressures relevant to tableting, including polymorphic transformations (e.g. chlorpropamide^{7,8}), partial amorphisation (e.g. theophylline, nitrofurantoin, amlodipine besylate⁹), and crystallisation of amorphous drugs (e.g. indomethacin, sucrose, celecoxib^{1,10}), using a range of characterisation techniques including differential scanning calorimetry (DSC), spectroscopy and X-ray and neutron diffraction. Investigations of pure substances have shown that the thermodynamic stability of polymorphs can change as a function of pressure¹¹ but, in addition, the type of pressure (isotropic vs anisotropic) is important and can promote or inhibit transformation, e.g. ammonium nitrate^{12,13}.

These changes have been observed not only for pure components but also within formulated tablets composed of drug-excipient mixtures to create robust compacts that mimic oral solid dosage forms for delivery to patients. In the context of tableting, phase transformations are induced through the exertion of an axial pressure on the powder which causes both normal and shear stresses. The shear stress is imparted by the friction between tablet surfaces and die wall and punches, which results in density gradients of the final tablet with denser regions closer to the surface⁸. Variables such as excipients, lubrication method, and compression conditions, e.g. pressure and dwell time, can all be explored to mitigate changes to the API solid form during this

process¹⁴. Temperature can also play a role with Infrared studies showing an increase of up to 10 °C post ejection^{15–19}.

However, once a tablet is formed, there are limited methods by which these tablets can be analysed at the crystal structure level. Phase transitions during compaction have required the break-up of the tablet and/or trituration of the powders and subsequent X-ray powder diffraction analysis to be able to observe the changes in the samples. Thakral *et al.*⁸ were able to reduce the sample preparation by cutting open the tablet and conducting 2D X-ray diffraction to map the changes across the tablet and spatially resolve polymorphic forms. This method still requires the need for sample preparation where changes to the product can occur leading to an uncertainty over the impact of the compaction process versus the subsequent treatment of the sample.

X-ray Computed Tomography (Xray-CT) is a method that has been used as a non-destructive analytical tool to investigate a wide range of research questions associated with pharmaceutical dosage forms. Using the variation in density, pressure gradient^{14,20}, coatings²¹, brittleness of components²² and agglomerates from crystallisation²³, the components of a dosage form can be spatially resolved. Interesting work evaluating the particle shape and how that can be used in X-ray-CT to spatially resolve polymorphs in a sample has been conducted and shown to 99% accuracy for clopidogrel bisulphate in a Plasdone S630 (N-vinyl-2-pyrrolidone and vinyl acetate) capsule²⁴. Several studies have explored the use of X-ray-CT to evaluate the impact of typical processes such as compaction²⁵ and dissolution^{26–29}. Specific to this study, Fu *et al.* used glass beads as tracers to evaluate the way in which a powder was compressed in a tablet die with force applied from a single direction. Using X-ray-CT the authors were able to follow the trajectory of glass tracer particles due to

the difference in density with the sugar spheres that made the bulk of the tablet and enabled the visualisation of the compression pattern. This study revealed that the distribution of compaction force was not uniform and that there was significant movement of powder on the side of the movable piston and interestingly virtually no movement around the base²⁵.

While these detailed studies have demonstrated that X-ray-CT is a powerful tool for visualisation, they require a contrast in the density of the solids or particle morphology to be able to resolve the solid mixtures, which is an issue in organic molecules where the density contrast between polymorphs, or even amorphous content, is very small^{30,31}. Critical to the resolution of this issue is crystallographic information. X-ray Diffraction Computed Tomography (XRD-CT) can provide this chemical and spatially resolved information, enabling the distribution of different components in a sample to be readily identified.

XRD-CT has been successfully applied to a number of material science areas to elucidate the composition of batteries³², catalysts³³, energy storage materials, and even biomineralisation in human bone structures³⁴. These studies have not only provided details under ambient conditions but also during working processes³³. These studies provide spatially-resolved physico-chemical information from within the interiors of intact objects negating the need for any intrusive sample preparation. In contrast to conventional absorption contrast tomography which uses a full-field approach to provide information on density/absorption differences within a sample, XRD-CT uses a pencil-beam scanning approach to measure the average diffraction signal through the sample at a given point. With the rotation of the sample and reconstruction of the

images from the data, a full crystallographic description of the sample that is spatially resolved can be provided³³.

A recent review of pharmaceutical tablets using X-ray tomography highlighted the lack of investigations using XRD-CT into these systems³¹. There is a significant challenge in this area due to the general low symmetry, large unit cell size, and poorer crystallinity often observed for softer organic materials. Here, this challenge is addressed and XRD-CT is applied to pharmaceutical tablets, which have been a significant omission as the field has developed. In particular, the evolution of polymorphic forms in pharmaceutical tablets as a function of compaction pressure is explored using glycolide as a pressure-sensitive marker.

Glycolide (Figure 5.1) undergoes a reconstructive phase transition at 150 MPa to a high-pressure polymorph (Form II) that is recoverable to ambient conditions, which makes it an ideal pressure marker for this study's *ex situ* measurements (Figure 5.2)^{35,36}. The pressure of this phase transformation is typical for an operating pressure in a tableting press used in the pharmaceutical industry. It should be noted that glycolide Form II has since been isolated at ambient pressure from cooling of a 2-propanol solution from 75 °C to 35 °C and is sufficiently stable to perform solubility measurements^{37,38}. Using XRD-CT, the impact of compaction pressure on the contents of the tablet has been visualised. These data indicate that the changes are far from uniform across the tablet. Serendipitously, the chemical degradation and subsequent crystallisation of the hydrolytic ring opening product of glycolide, 2-(2-hydroxyacetoxy)acetic acid, has been observed *in situ* within a tablet on storage, which has not been observed to date.

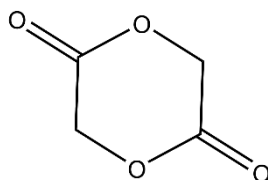


Figure 5.1. Molecular structure of glycolide.

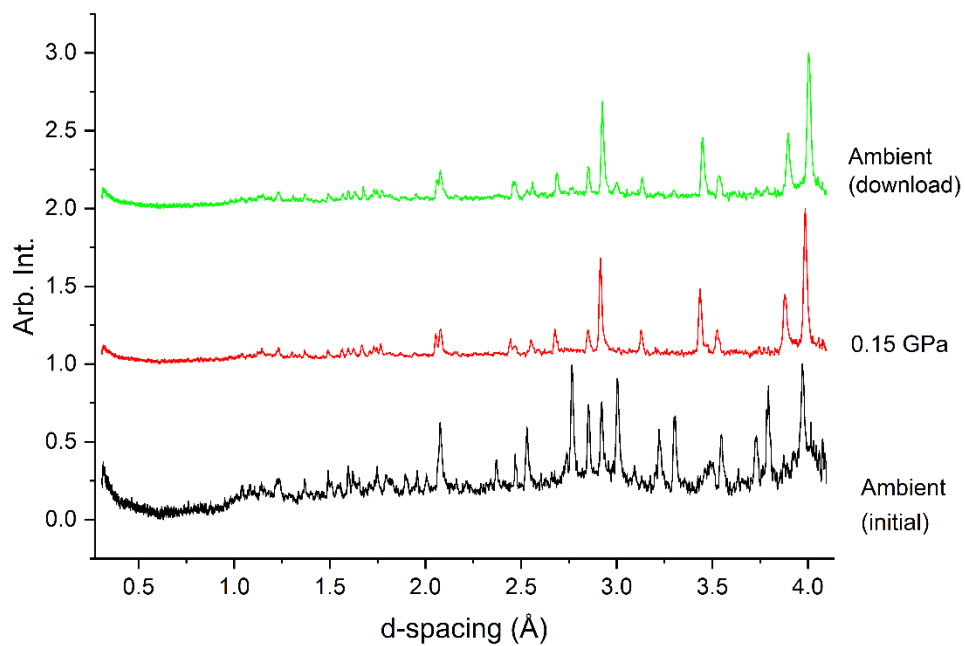


Figure 5.2. Neutron powder diffraction patterns of glycolide at ambient and 150 MPa and its recovery to ambient pressure. (Reproduced with permission of the International Union of Crystallography)³⁶.

5.3. Materials and Methods

5.3.1. Materials

Glycolide (1,4-dioxane-2,5-dione, 97%, CAS no. 502-97-6) was purchased from Thermo Fisher Scientific UK as a polycrystalline powder. VIVAPUR® 112 microcrystalline cellulose (MCC), purchased from JRS Pharma (CAS no. 9004-34-6), was used as filler. VIVAPUR® PH-112 is a commercial grade of MCC not typically used in direct compression formulations. However, its low moisture content helps reduce the occurrence of water-sensitive changes in the formulation. Technical grade magnesium stearate (MgSt), purchased from Sigma Aldrich UK (CAS no. 557-04-0), was added as an internal lubricant to prevent sticking during compression. Both excipients were used without further treatment. All the materials used in this formulation are hygroscopic.

5.3.2. Particle size reduction

Glycolide was cryomilled using a Retsch CryoMill with a 25 mL stainless steel grinding jar and a single 15 mm diameter stainless steel grinding ball. Glycolide (2.00 g) was pre-cooled and milled at -196 °C (i.e., under a constant flow of liquid nitrogen from a 50 L Dewar). The material was pre-cooled for 5 min. at 5 Hz, then milled for 9 cycles of 5 min. at 25 Hz, with each milling cycle alternated with a 5 min. cycle at 5 Hz to prevent overheating of the sample. Once micronised and before blending, the material obtained was stored in an airtight container at 5 °C to prevent chemical degradation.

5.3.3. Powder characterisation

The phase purity of glycolide (both pre- and post-micronisation), MCC and MgSt was checked and confirmed in-house using powder X-ray diffraction (XRPD). Samples of glycolide were not ground prior to analysis to prevent triggering the phase transition. XRPD data of a small quantity of sample (10 – 50 mg) was collected at ambient temperature on a Bruker D8 Discover diffractometer, configured in a Debye-Scherrer transmission geometry and equipped with a SSD160-2 1D detector and an automated multiposition x-y sample stage. The sample was placed on a 40 position well plate lined with a 7.5 μm Kapton film and was scanned across a 4 – 40° 2 θ range with a 0.017° 2 θ step size and a 0.5 s per step count time. Samples were oscillated in the x-y plane at a speed of 0.03 mm/s throughout data collection to maximise powder sampling and minimise preferred orientation effects. X-ray emission was supplied by a Cu source ($\lambda = 1.54060 \text{ \AA}$, sealed tube operated at 40 kV and 10 mA) paired with a $\text{K}\alpha_{1,2}$ primary focusing Göebel mirror monochromator.

5.3.4. Tablet formulation

5.3.4.1 Tablet preparation

Before blending, micronised glycolide was passed through a stainless steel sieve with a 180 μm aperture to remove any large or agglomerated particles. The sieved glycolide (5 g) was mixed with MCC (19.75 g) by manual geometric blending. Both powders were further mixed using a Pharmatech MultiBlend® MB015 bench top powder blender to ensure powder blend homogeneity, using the following parameters: set blend speed of 20 rpm, set agitator speed of 100 rpm, set blend time of 25 min., set agitator time of 24 min. and delay for agitator of 30 sec. As a final step, MgSt (0.25 g) was sprinkled over the mixture, which was manually tumbled in perpendicular

directions 10 times to prevent overmixing and segregation of MgSt. The final powder blend contained glycolide, MCC and MgSt in a 20:79:1 %w/w ratio. During tablet preparation, tablets of the same thickness were compressed at different pressures by applying a 2.15 mm punch separation during compaction and varying the weight of the tablets. The manual method of die fill resulted in a variance in applied pressures defined in the “Tablet name” column in Table C5.3.

5.3.4.2 Tablet storage

A DS1923 Hygrochron iButton Temperature/Humidity logger (Measurement Systems Ltd, Berkshire, UK) was used to monitor temperature and humidity during storage. It was stored inside a sealed petri dish, mimicking a sealed container, and recorded the temperature ($\pm 0.5^{\circ}\text{C}$) and humidity ($\pm 5\%\text{RH}$) every 600 seconds for approximately 3 days.

5.3.5. X-ray diffraction computed tomography

5.3.5.1 Fresh samples

XRD-CT measurements on freshly prepared tablets were performed using EH2 of beamline P07 at the PETRA III synchrotron at DESY. A monochromatic X-ray beam of 74 keV (0.168 Å) was focused to have a spot size of $10 \times 1 \mu\text{m}$ (Horizontal x Vertical). 2D powder diffraction patterns were collected at 100Hz using a Pilatus3 X CdTe 2M hybrid photon counting area detector. The tablets were mounted edge-on onto a goniometer (such that the scan would pass through the tablet top-bottom) which was placed on a rotation stage which was mounted perpendicularly to a hexapod; the hexapod was used to translate the sample across the beam. The XRD-CT scans were measured by performing a series of zigzag line scans in the z (vertical) direction using the hexapod and rotation steps. 600 translation steps and 600 rotation steps over 0-

360° range were used per sample with a translation step size of 10 µm and 10ms collection time per point.

5.3.5.2 Aged samples

XRD-CT measurements on the same set of tablets aged for 1 month were performed on beamline ID31 of ESRF. Here, the monochromatic X-ray beam energy was 80 keV, focused to a spot size of 20 x 3 µm, and the data was collected at 125 Hz using a Pilatus3 X CdTe 2M hybrid photon counting area detector. 600 translation steps of 10 µm, 10ms collection time per point and 600 rotation steps over 0-360° range were used per sample.

5.3.5.3 Data collection and analysis

For both sets of beamtime data the detector calibration was performed using a CeO₂ standard, with each 2D diffraction image being calibrated and azimuthally integrated to a 1D powder diffraction pattern, both without filtering and with mean-trimmed filters applied at 5% and 10% using the pyFAI software package and in-house developed scripts^{39,40}.

The integrated diffraction patterns were reshaped into sinograms and centred. The XRD-CT images (i.e., reconstructed data volume) were reconstructed using the filtered back projection algorithm. The voxel size in the reconstructed images corresponds to 10 × 10 × 1 µm³ for the data collected on P07 at DESY and 10 x 10 x 3 µm³ for data collected on ID31 at the ESRF. To aid interpretation of the data the scattering axis was rescaled to that of a standard lab diffractometer using a Cu Kα source (8.047 keV, 1.5406 Å). Phase identification was performed using Mercury⁴¹ and X'pert Highscore Plus. The simulated data for the identification of phases was taken from: X'pert database (Magnesium stearate, 00-005-0292), Cambridge Structural Database entries

(glycolide I and II; NAHNIT01, NAHNIT02; cellulose 1 α and 1 β ; JINROO05, JINROO01)^{35,42}. Maps of each phase were generated using unique reflections, and composite images created using ImageJ⁴³.

5.3.6. Temporal stability study

Loose powder samples of micronised pure phase glycolide and a blended mixture of glycolide, MCC and MgSt in a 20:79:1 %w/w ratio were prepared following the methods described in Sections 5.3.2 and 5.3.4.1. The samples were tested 1, 7, 14, 21, 28, 35, 42, 47, 56, and 302 days after being prepared, using powder X-ray diffraction and following the method described in Section 5.3.3. The 40-position well plate was lined with two 7.5 μ m Kapton films, one above and one below the samples, in order to seal the samples within the plate and minimise their interaction with atmospheric water.

5.3.6.1 Electron diffraction

The sample of 2-(2-hydroxyacetoxy)acetic acid was finely dispersed on a standard transmission electron microscopy (TEM) grid (amorphous carbon on Cu) without grinding and measured on an ELDICO ED-1 electron diffractometer at room temperature using the software ELDIX⁴⁴. The device is equipped with a LaB6 source operating at an acceleration voltage of 160 kV ($\lambda = 0.02851$ Å) and a hybrid-pixel detector (Dectris QUADRO). Suitable crystals were identified in STEM (scanning transmission electron microscopy) imaging mode (Figure C5.15) and diffraction was recorded in continuous rotation mode with a beam diameter of ca. 750 nm. Parts of measurements showing significant beam damage or shadowing by the grid were omitted. Further data collection details are given in Table 5.1.

Table 5.1. ED data collection details for the crystals of 2-(2-hydroxyacetoxy)acetic acid used in refinement.

Crystal no.	Angular range [°]	Rotation per frame [°]	Exposure time [s]	Total exposure [s]	Frames measured	Frames used
1	−60 to +60	1	0.5	120	120	1 to 120
2	−60 to +60	1	0.5	120	120	1 to 55
3	−60 to +60	1	0.5	120	120	1 to 120
4	−60 to +60	1	0.5	120	120	11 to 110
5	−65 to +65	1	0.5	130	130	1 to 50
6	−55 to +55	1	0.5	110	110	1 to 50

Data were processed and evaluated using the APEX4 software package⁴⁵. After unit cell determination the frames were integrated separately for each crystal, then merged, scaled, and corrected for Lorentz effects, scan speed, background, and absorption using SAINT and SADABS^{46,47}. Space group determination was based on systematic absences, E statistics, and successful refinement of the structure. The structure was solved using ShelXT and refined with ShelXL in conjunction with ShelXle^{48–50}. Least squares refinements were carried out within the kinematic approximation by minimising $\sum w(F_{\text{obs}}^2 - F_{\text{calc}}^2)^2$ with the ShelXL weighting scheme and using neutral electron scattering factors^{49,51}. H atoms bound to C were placed in calculated positions based on typical distances for neutron diffraction and refined with a riding model and $U_{\text{iso}}(\text{H}) = 1.2 \cdot U_{\text{eq}}(\text{C})$. H atom positions on heteroatoms were refined freely.

5.4. Results and Discussion

5.4.1. Initial examination of the tablets

The initial structural investigations^{35,36} of glycolide were performed on the pure material itself using a liquid pressure-transmitting medium to ensure that the structural information could be extracted under hydrostatic or pseudohydrostatic conditions. These conditions are not typical in a tableting environment, hence it was necessary to demonstrate that the transition would still occur in the presence of excipients and under conditions of tableting. An initial test was performed on an in-house compaction simulator (HB50, Huxley Bertram Engineering Ltd) and using a terahertz time-domain spectroscopy (THz-TDS) system (TeraPulse Lx Spectrometer, TeraView Ltd). A formulation containing glycolide (20%wt.), microcrystalline cellulose (VIVAPUR® PH-112; 79%wt.) and magnesium stearate (1%wt.) was loaded into the compaction simulator before applying incremental load and characterising tablets offline using the THz-TDS system. The resulting spectra enable the measurement of the absorption coefficient of the solids on increasing pressure hence it can be inferred that an observable discontinuous change can be attributed to a phase transformation in the material of study⁵². There is a clear discontinuous change to the spectra on compression above 150 MPa even in the presence of the excipients (Figure 5.3) warranting further investigation using XRD-CT.

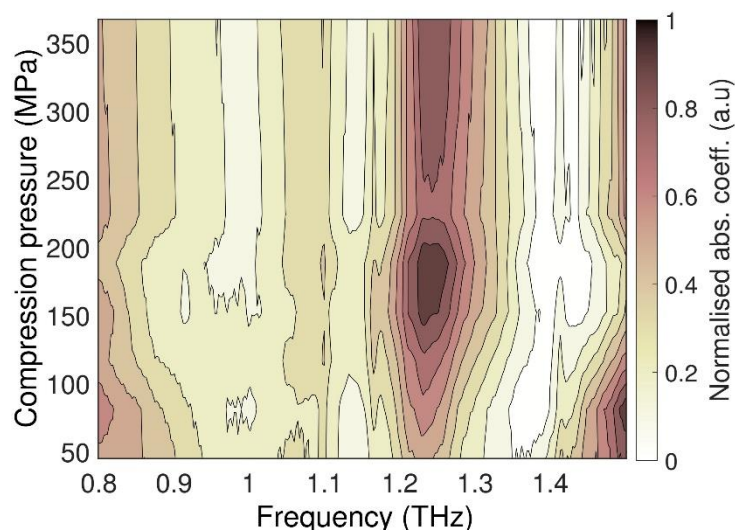


Figure 5.3. Terahertz time-domain spectroscopy (THz-TDS) of a tablet of glycolide. A change in absorption coefficient at 150 MPa indicates the phase transition has occurred.

To evaluate the effect of compaction pressure and obtain structural information on the degree of polymorphic transformation in glycolide tablets, 47 tablets were compressed to pressures ranging from 47 to 548 MPa with 20 tablets used for analysis. Blended powders were weighed and manually loaded into the die to compress the tablets to target pressures of 50, 100, 130, 150, 170, 200, 300, 400 and 548 MPa (Table C5.3). The weight of the tablet was altered to ensure that the tablet thickness was constant while varying the compaction pressure. Flat-faced round steel punches (diameter: 5 mm) were used to compress the powder over a 1 s period using a uniaxial compression profile. The tablets were prepared days in advance of characterisation on beamline P07- DESY at PETRA III⁵³. Between preparation and characterisation, the tablets were stored in a sealed container at $5^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $65\% \pm 5\%$ relative humidity. The tablets underwent full relaxation before being studied⁵⁴.

The tablets were mounted onto a standard Huber goniometer in two orientations to provide lateral and longitudinal cross sections (Figure 5.4a,c) using the XRD-CT

method which typically takes ca. 1 hour for the data collection. Phase identification of the reconstructed XRD-CT datasets was performed on summed global patterns using X'Pert HighScore Plus⁵⁵, with the phases identified as being glycolide Form I (CSD refcode NAHNIT01), glycolide Form II (CSD refcode NAHNIT02), microcrystalline cellulose and an unknown phase, later identified to be 2-(2- hydroxyacetoxy)acetic acid. Magnesium stearate was added at a low level of 1% wt. in the powder blend so its presence in the tablets was sparsely detected. Unique reflections were identified and used to generate the spatial distribution maps of each phase.

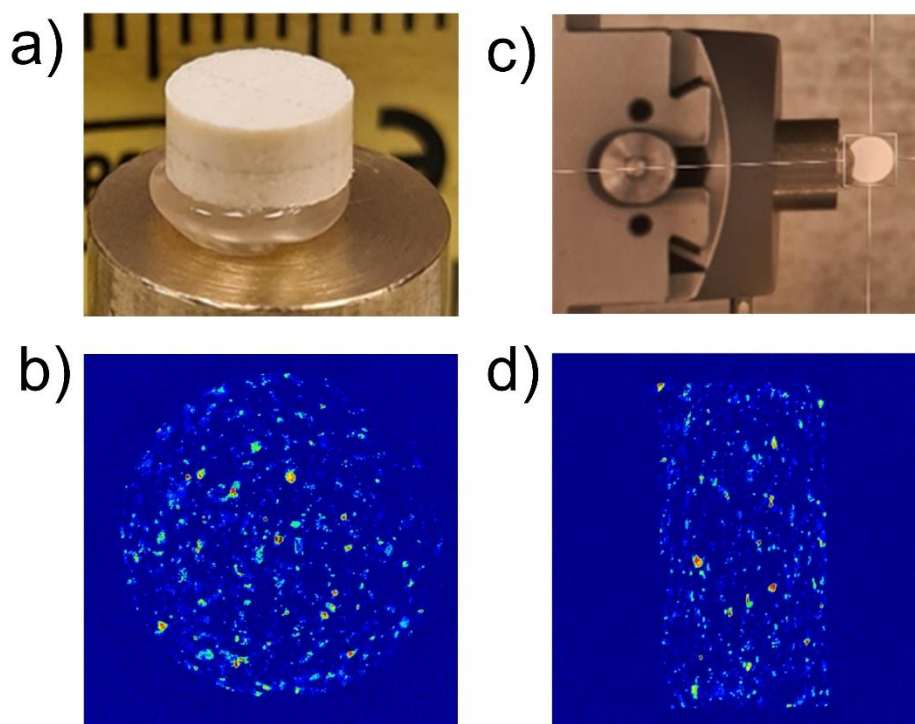


Figure 5.4. (a) Example tablet (not glycolide tablet) mounted on the goniometer at P07-DESY at PETRA III. The dark lines on the tablet indicate the beam path during experiment; (b) XRD-CT image of a tablet as orientation in a). The colour scheme indicates the presence of the Form I in the tablet (blue-red from absence to presence). (c) second orientation of the tablet indicating where the tablet was glued to the mount; (d) image of the tablet using colour map of presence of Form I; same colour scheme to (b)).

5.4.2. Mapping the high-pressure phase of glycolide

A selection of tablets across the 47 to 548 MPa range of compaction pressures were subject to analysis and their composition maps created. The discussion has been limited to the side profile of the tablet, shown in Figure 5.4d, however, similar observations are found for the front-facing direction. The reconstructed images of the tablets (Figure 5.4d) clearly show the spatial distribution of the phases, where normalised glycolide Form I distribution is represented by a jet colour Scheme. The diffraction patterns indicate that, of the known forms of cellulose, the MCC present can be identified as Cellulose I⁵⁶. Cellulose I can be split into two crystallographically independent phases, I α and I β ^{57–59}; polymorph I β is the more thermodynamically stable form⁶⁰. Figure 5.5 shows the XRD-CT images showing distribution of Form I and II for tablets compressed at different pressures. In the low pressure image of 47 MPa, it shows that the components of the tablet are homogeneously mixed together with an even distribution of the Glycolide Form I in the bulk MCC. MCC appears to remain unaffected and its distribution is consistently maintained across all pressures (Figure C5.12) which supports previous observations^{61,62} hence the discussion will focus on the transformations in glycolide (Figure 5.5a).

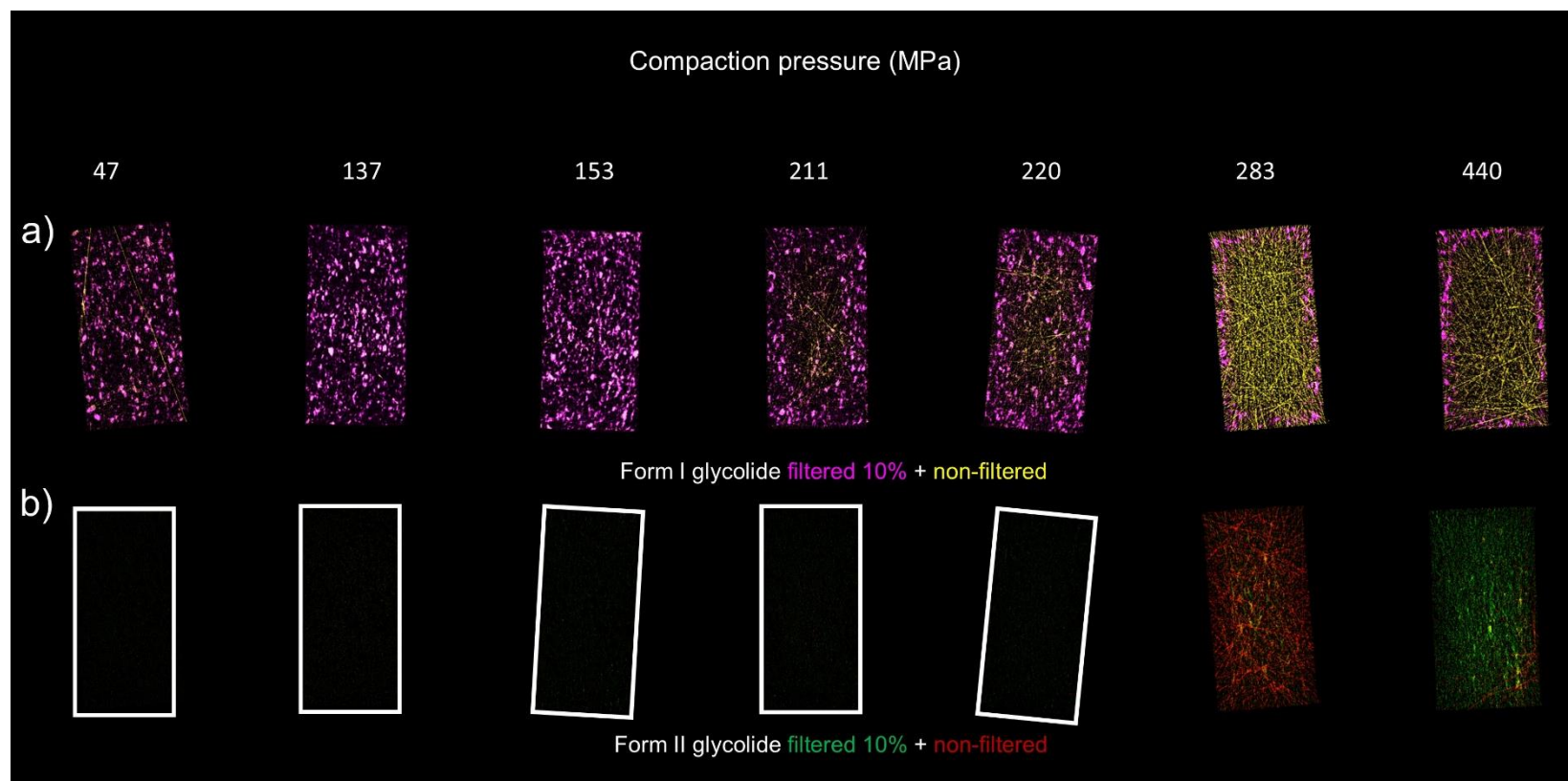


Figure 5.5. XRD-CT images of tablets compacted at different pressures. (a) Form I glycolide filtered 10 % (pink) and non-filtered (yellow). (b) Form II glycolide filtered 10 % (green) and non-filtered (red). The images for pressure points 47–220 have been given borders to outline the edge of the tablets due to the scale of the data.

A key consideration in XRD-CT is the way in which data are treated. XRD-CT requires good, well-distributed powders so that the reconstruction is not impacted by diffraction events from single crystals. These events manifest themselves in “streak” artefacts in the reconstructed images and distort them, hence an approach to counter this without compromising the information in the patterns is needed³⁹. Organic materials, including glycolide, can be poor powders with larger particles present despite significant particle size reduction through milling. Typically, this problem is addressed via a mean-trimmed filtering approach which removes values above/below a specified limit, e.g. the top and bottom 10% of diffraction intensity at any given 2θ value. By this approach, the significant individual spots are removed whilst, crucially, maintaining the diffraction from the rest of the powder at the same 2θ range. The omitted data lie on the azimuthal rings at the same 2θ values as the “good” powder rings, which have an even distribution of intensity around the ring; trimming therefore removes the uneven single-crystal contributions without discarding the underlying powder signal. This process leads to significantly improved image quality in the reconstructions (Figure 5.6).

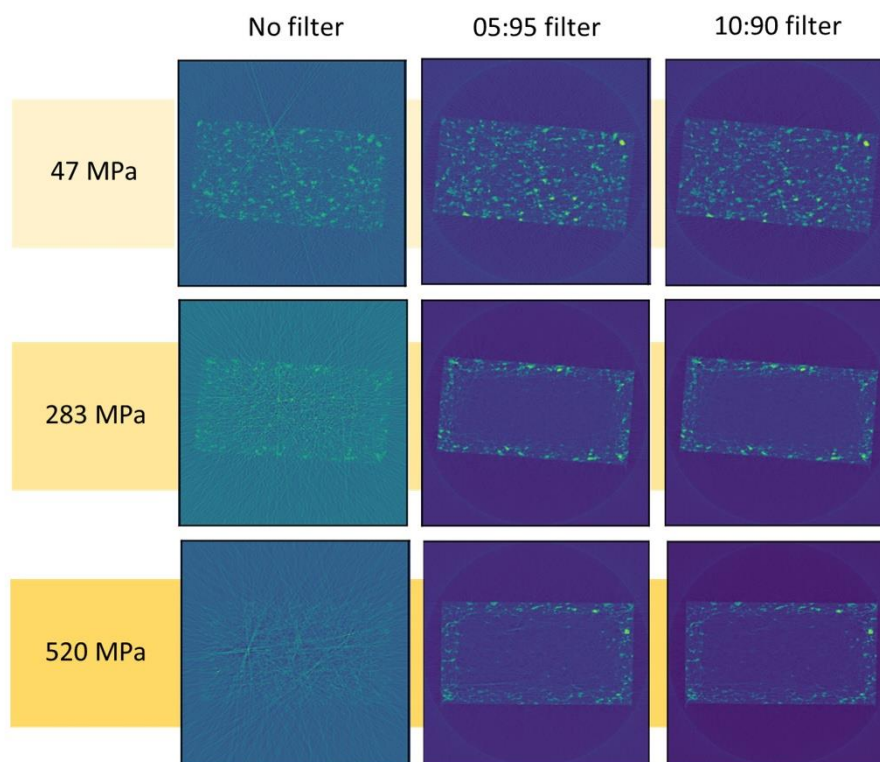


Figure 5.6. The images represent the various stages of mean-trimmed filtering at different levels using tablets at different compaction pressures. The data represented here is using the 2-theta values of Form I of glycolide (CSD refcode: NAHNIT01³⁵).

Consideration of the filtered data alone provides clear distributions in the reconstructed images (Form I, pink; Form II, green; Figure 5.5). As the compaction pressure is increased, changes to the intensity and distribution of Form I become apparent (pink, Figure 5.5a). The observed intensities of Form I diminish in the centre of tablets compressed to higher pressures but are present at the edges of the tablet. This change clearly begins between 153 and 211 MPa. At the same time, the contribution from Form II is not significant until 283 MPa (green). The pressure at which the changes occur is commensurate with the previous diffraction studies but there is a difference between the pressures at which these events occur indicating that they cannot be correlated. To help explain this it is necessary to consider the non-filtered

data as this reveals some significant findings to enhance our understanding of the changes.

5.4.3. Change in the diffracting object size of glycolide upon compaction

The non-filtered data (yellow, Form I; red Form II) reveals an increase in the presence of larger crystallites in the centre of the tablet, those for Form I start to appear at 211 MPa reaching a maximum at 283 MPa before diminishing at 440 MPa. This indicates that at higher compaction pressures the original powder particles are annealing together to form larger particles when subjected to compression. Glycolide is a van der Waals solid which may facilitate this process due to the weaker, more malleable intermolecular interactions. This phenomenon is similar to the behaviour observed in other van der Waals solids like benzene when solidified under pressure^{63,64}. Particle agglomeration, dominated by solid bridge formation, has also been reported during the compression of micronised small-molecule APIs like furosemide and griseofulvin blended with excipients⁶⁵. Although the author did not discuss the single-crystal-like nature of the agglomerates, Potharaju noted that the agglomerate size increased steadily with increasing compression force, paralleling the observed trend for glycolide Form I.

5.4.4. Transition to Form II and its retention

The question remains, is the transition to Form II observable in tablets compressed to pressures in excess of 150 MPa as was proposed? From the data, a stark change to the diffraction attributable to Form II is observed at 283 MPa and beyond. At 283 MPa, there is an increase in the non-filtered powder diffraction for Form II (red, Figure 5.5b) which may be accounted for by the transformation of the larger annealed crystals from Form I to Form II. The compaction pressure at which the changes are observed

correlates reasonably well with those from the initial neutron diffraction and terahertz spectroscopy experiments³⁶. At higher tableting pressures (440 MPa) less streaking for both Form I and II is observed, suggesting that the particles undergo fragmentation into smaller particles providing better powder diffraction. There is clear evidence of a greater proportion of Form II across the tablet at this higher compaction pressure (more intense colour Figure 5.5b).

From the observations, there is approximately a 50 MPa difference between the point where a change in the distribution of Form I is evident, and the phase transition pressure of glycolide under hydrostatic conditions. For a pure glycolide compact, a lower phase transition pressure would be anticipated upon compaction, given the reduced size of glycolide particles³⁶ and the increase in shear stress upon anisotropic compression. Thakral *et al.* observed that when chlorpropamide in a blend with a significant MCC content (50 : 50 wt%), the presence of MCC reduced the extent of transformation between polymorphs⁸. Though the reduction observed in the Thakral study was subtle, a more pronounced impact of the formulation on transformation pressure is reported here, as it contains a higher MCC content (80% wt). Li *et al.* have also shown that magnesium stearate presence in blends can also alter the compaction pressure required to induce a phase transformation^{8,66}.

One of the key observations of this study is that the retention of Form II on decompression has enabled the impact of pressure on the system to be observed without the destruction of the tablet. Form II is still the thermodynamically less stable form under ambient conditions, hence there is a driving force to transform back to Form I. The local environment, i.e. whether it is in the centre or the edge of the tablet, will play a role in the kinetics of the transition and this was observed in the image of

Form I around the edge at 211 MPa. Those crystallites at the edges of the tablet are much more likely to convert to Form I due to the interaction with moisture in the air and also through shear force interaction with the die holder of the tablet press. Both of these interactions can facilitate the transition back to the ambient form^{67,68}. The Form I to Form II transition is reconstructive, where bigger particles are broken up into smaller particles⁶⁹, even if micron-sized. A result of this is that the powder produced should become a powder with improved diffraction statistics by reducing any preferred orientation that may be present initially. Both of these factors aid the analysis using XRD-CT as there is less requirement to filter out single crystal diffraction providing a more optimal reconstruction of the image. This is observed through a reduced number of single crystal particles around the edges of the tablet and the increased resolution of the border around the edge of the tablet as increasing compaction pressure is used.

5.4.5. Glycolide stability as a function of time

Due to the allocation of synchrotron beamtime, the effect of storage time on the same tablets could be explored to observe whether the conversion back to Form I would occur. Stability testing of pharmaceutical products is a critical component of the drug development process. As part of this study, drug-excipient compatibility⁶⁹ was monitored as a function of time by collecting data one month after the initial compaction. Where possible, the same tablets that were used initially were re-examined but, in some cases, this was not possible due to the tablet fracturing while unmounting the sample from the goniometer. The tablets were stored under the same low temperature and high relative humidity conditions. Overall, visually, the samples showed a significant increase in the roughness of the overall tablet surface upon 1 month of storage.

XRD-CT of all the samples measured indicates a significant change to the structure of the components of the tablets (Figure 5.8a). Firstly, both forms of crystalline glycolide deteriorated over the course of a month as there was no indication of either Form in the tablet. Secondly, the peak height and sharpness of the diffraction pattern of an unknown phase were both enhanced in the aged samples indicating increased formation of this crystalline solid during storage, as seen in Figure 5.8a for two different compaction pressures. Interestingly, additional diffraction patterns isolated from parts of the tablet cannot be identified as any of the known forms of any of the components. In these images, the white areas represent the distribution of the unknown phase; a comparison of the global diffraction pattern with the respective phase is shown in Figure 5.7. It can clearly be observed that there is a small quantity of this phase in the fresh tablets, but the ageing has caused a significant increase throughout the tablet. It is reasonable to assume from the extent of observation that it is related to either the glycolide or the MCC content based on the initial weights in the tablet.

The global pattern from the aged tablet compressed to 440 MPa and was deconvoluted into the component parts (Figure 5.7). From this pattern, the unknown phase is the largest component in combination with the broader underlying MCC phase. There is a smaller contribution from glycolide Form II but there is a further phase that was unidentified. Key Bragg diffraction peaks between 16.55 and 16.95 were not associated with the known phases. Due to the larger proportion this phase in the aged tablets, it was initially thought that the MCC had recrystallised into Cellulose 1 β due to the proximity of the peaks at 14.86° and also 16.66°. The recrystallisation of MCC is known, however this would be highly unusual as MCC is used extensively as a bulking agent.

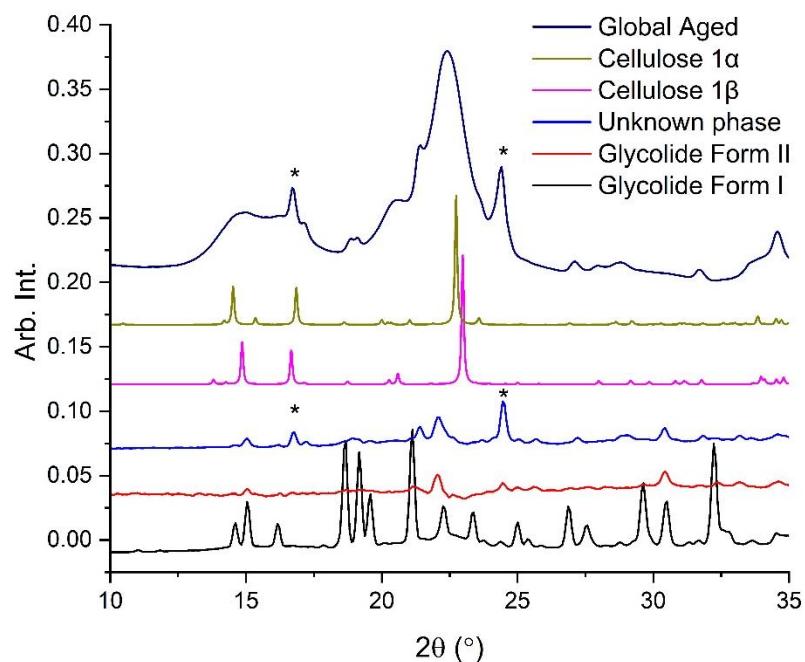


Figure 5.7. Similarities between peak positions in the X-ray diffraction patterns of glycolide Form I, Cellulose I α , Cellulose I β and the unknown phase.

Considering MCC initially, the diffraction pattern shows that Cellulose I^{57,58,70,71} is present as a mixture of the two different polymorphs, I α and I β ^{57,58,60}. The unknown phase has a sharper diffraction pattern from a more crystalline product indicating conversion to either one of the two polymorphs. Cellulose I β is the more likely choice due its higher stability (approx. 8 kJ mol⁻¹^{59,60,72}) and the pair of Bragg reflection above $2\theta = 16^\circ$ and those above $2\theta = 20^\circ$ are somewhat more discriminatory (Figure 5.7). Conversion of MCC during storage has not been observed during stability studies^{62,73,74} and the only transition reported from I α to I β was after an annealing process at 260 °C using NMR as a probe⁷⁵ hence it is unlikely that the new material has its origin from MCC.

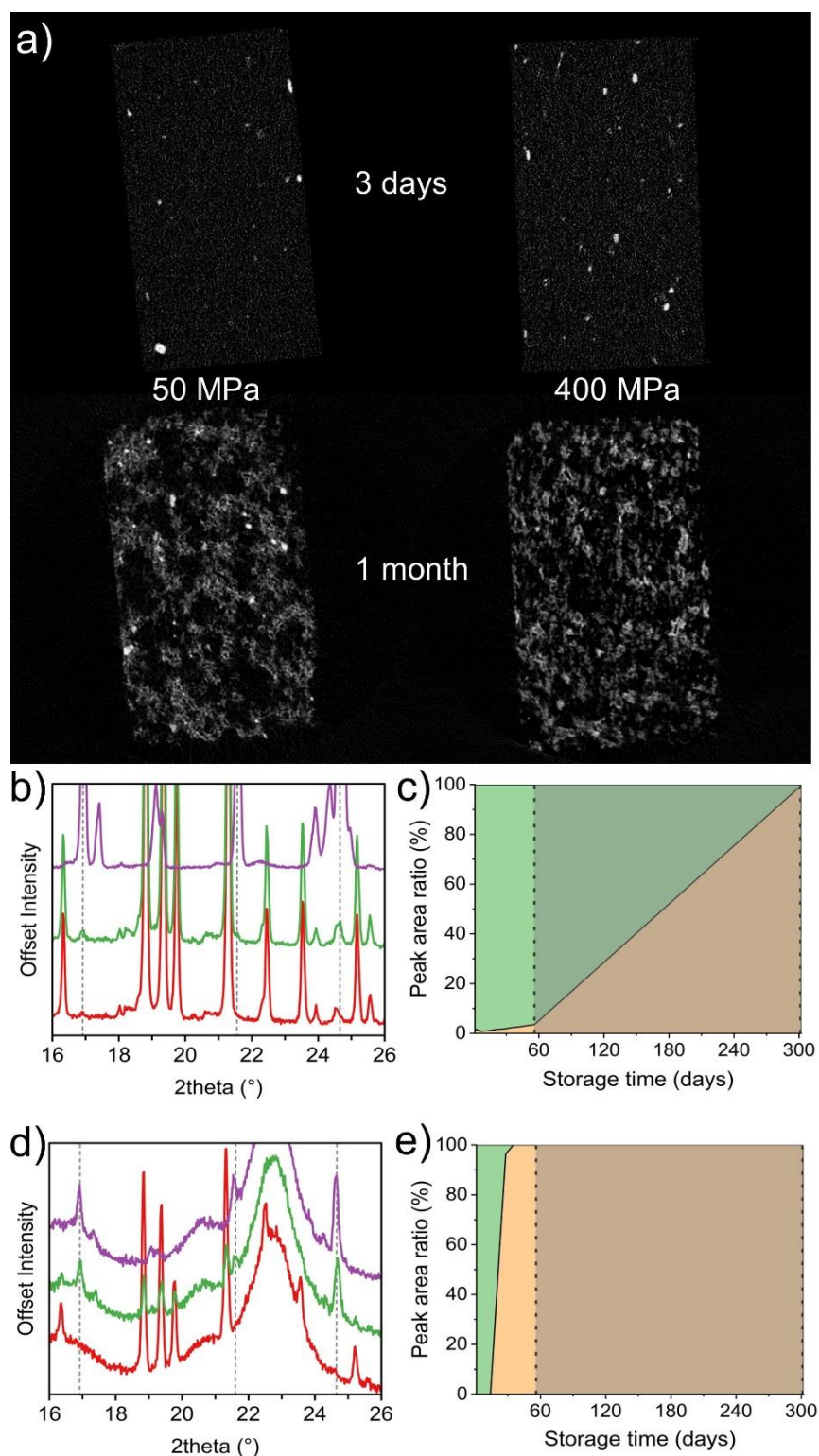


Figure 5.8. (a) X-ray Diffraction CT images for the tablets compressed to 50 MPa and 400 MPa at $t=4$ days (fresh) and $t=30$ days (aged). The white particles indicate the presence of 2-(2-hydroxyacetoxy)acetic acid. The XRPD patterns of the pure (b) and blended (d) mixtures are shown for day one (red), three weeks (green) and 10 months (purple) of storage. The change in the area under two characteristic peaks in the XRPD patterns of glycolide Form I ([002]; green area) and 2-(2-hydroxyacetoxy)acetic acid ([100]; orange area) is tracked in the pure (c) and blended (e) mixtures over time and compared as a ratio. The equivalent analysis across other pairings of characteristic peaks is given in Figure C5.14.

The disappearance of the two forms of glycolide suggests that the unknown product is related to this either as a new solid-state form of glycolide e.g. polymorph, hydrate etc. or a reaction product. Separate time-dependent stability studies of glycolide powder were performed in-house. Glycolide was stored as the pure phase and as the blended mixture under the same conditions as the tablets used in the study (5 °C and 65% RH). The powder diffraction study (Figure 5.8b-e) shows that glycolide converts to the same unknown phase over the course of a few weeks. In both the pure and blended mixtures, the conversion to the unknown phase begins after two to three weeks but the extent of conversion appears to be accelerated in the blended product with complete conversion after four weeks where the conversion of the pure phase is complete after 10 months. No data were collected between 2 and 10 months due to assumed stability but 3.6% conversion had occurred at 2 months in the pure sample. The conversion of the pure phase enabled indexing of the sample with unit cell parameters of $a = 5.28(5) \text{ \AA}$, $b = 5.35(5) \text{ \AA}$, $c = 19.92(19) \text{ \AA}$, $\beta = 90.900(13)^\circ$, volume = $562(9) \text{ \AA}^3$ and space group $P2_1/c$.

The percentage conversion based on the integration of selected Bragg peaks that are representative of each phase is summarised in Figure C5.14 (glycolide (002) at 18.68° & (012) at 19.62° , HAA [100] at 16.77° & [011] at 17.22°). One of the more intriguing elements is that there is transformation of glycolide in its pure state. Storage of unmanipulated glycolide does not appear to transform (Figure C5.10; purple and blue patterns) so it suggests that the micronisation process of glycolide initiates this to occur. Glycolide was cryomilled to reduce the particle size for the analysis using XRD-CT hence there was potential that some condensation will have occurred in the vessel

that might promote the reaction. There is evidence of the HAA solid in the micronised product at a low level (Figure C5.11).

Unfortunately, the structure could not be solved from the powder diffraction data but the crystal quality was good enough to use the recent developments in electron diffraction to solve the crystal structure of the unknown phase^{76,77}.

5.4.6. Uncovering the formation of 2-(2- hydroxyacetoxy)acetic acid

The unknown phase from the stability studies is 2-(2- hydroxyacetoxy)acetic acid (HAA), the hydrolytic ring opening product of glycolide (Figure 5.9). The crystal structure from the ED data provides an excellent fit to the XRPD patterns collected as part of the stability study and as tablet (Table 5.2), confirming phase purity (Figure C5.16). HAA crystallises in the monoclinic space group $P2_1/c$ with one molecule in the asymmetric unit. The molecules form interactions primarily through the carboxylic acid $R_2^2(8)$ dimer ($O1\cdots O2$; 2.696(19) Å). At the other end of the molecule the hydroxyl group (O5) is involved in a bifurcated hydrogen bond to O4 and O5 of a neighbouring molecule (2.90(2) Å). The combination of the interactions provides a three-dimensional hydrogen-bonded network with a layered herringbone structure (Figure 5.9). To the best of our knowledge, this is the first time this structure has been reported.

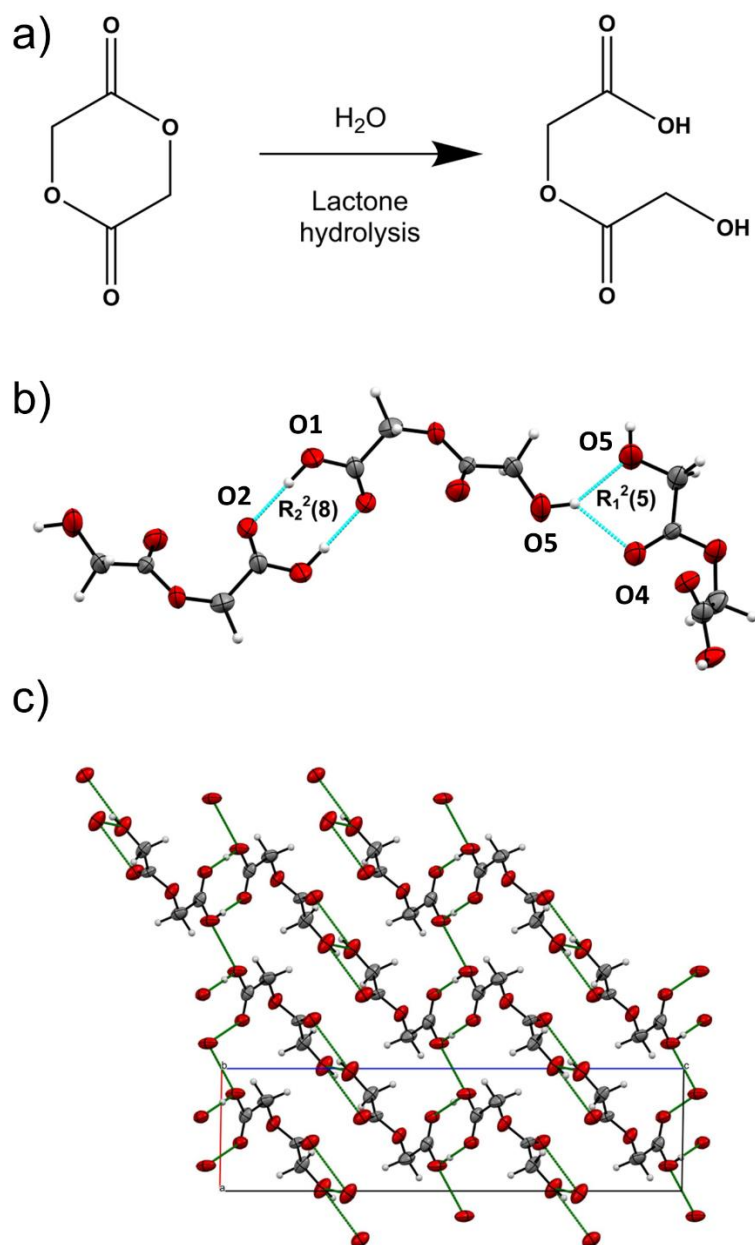


Figure 5.9. (a) Lactone hydrolysis of glycolide to 2-(2-hydroxyacetoxy)acetic acid; (b) hydrogen bond motifs in the crystal structure of 2-(2-hydroxyacetoxy)acetic acid, indicating the carboxylic dimer $R_2^2(8)$ and the bifurcated H-bond between the hydroxyl group and the carbonyl groups ($R_1^2(5)$); (c) the packing of the molecules showing the three-dimensional bonding of the structure.

Table 5.2. Crystallographic summary for 2-(2-hydroxyacetoxy)acetic acid. Full data collection and refinement details are given in Appendix Table C5.4.

	Electron diffraction	Rietveld powder
Chemical formula	C ₄ H ₆ O ₅	C ₄ H ₆ O ₅
M_r	134.09	134.09
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
Z	4	4
a (Å)	5.28(5)	5.28703(18)
b (Å)	5.35(5)	5.33231(16)
c (Å)	19.92(19)	19.8459(7)
β (°)	90.900(13)	90.612(2)
V (Å ³)	562(9)	559.46(3)
Temperature (K)	298	298
Radiation type	$\lambda = 0.02851$ Å (electrons)	$\lambda = 1.54043$ Å (X-rays)

5.4.7. Role of moisture in glycolide hydrolysis

Having determined the source of the new diffraction data, the process of the conversion between glycolide and HAA can begin to be understood. It is postulated that the hygroscopic nature of MCC increases the moisture content within the blend to drive and increase the rate of hydrolysis. Suzuki and Nakagami⁷³ showed that MCC with a higher amorphous content was more hygroscopic than with a higher crystalline content (25% vs. 65% crystallinity) with an ~8% increase in moisture uptake for the higher amorphous content MCC. In general, cellulose has a crystallinity that varies between 53–82%^{78,79} depending on the source of the MCC. In addition, glycolide is

hygroscopic itself which will enhance the uptake of moisture hence conversion was observed in the pure phase as well, albeit more slowly. The stored tablets appeared to have a rougher surface compared to their initial state which can be accounted for by the recrystallisation of HAA as the hydrolysis reaction occurred. The low temperature storage conditions and slower rate of crystal growth will have contributed to the production of a highly crystalline powder. Over this time the presence of MCC within the tablets does not change hence the degradation of the glycolide does not appear to have affected the MCC. This observation is supported by previous investigations into various grades of MCC using X-ray diffraction, which did not show any significant change to the diffraction pattern despite being subject to various environments⁶².

5.5. Conclusions

This study showcases the effective utilisation of XRD-CT methodology in spatially resolving components with similar densities within pharmaceutical tablets, offering a detailed map of their composition. Despite the challenges posed by deciphering diffraction patterns of low symmetry systems and low molecular weight, phase transitions were successfully observed directly in these tablets without resorting to sample manipulation such as trituration of the tablet into powder, thus avoiding potential polymorphic transitions in the compounds. By employing a pressure-sensitive compound, the precise locations of phase transitions were identified and the impact of compaction pressure on formulation was assessed. Notably, this approach has enabled both temporal and spatial resolution of changes occurring in these tablets. Furthermore, this investigation serendipitously uncovered a solid-state chemical reaction within the tablet, wherein the hydrolytic ring opening product of glycolide, 2-(2-hydroxyacetoxycarboxylic acid (HAA), was first identified using XRD-CT and subsequently confirmed through electron diffraction, culminating in the elucidation of its crystal structure. In the wider context of pharmaceutical research, the application of this technique to pharmaceutically relevant formulations enables a deeper, more intimate understanding of the effect of tableting pressure on the formulation in pharmaceutical products. The molecular level of insight, without the destruction of the sample, is a step change in the way in which formulated products can be viewed.

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Appendix

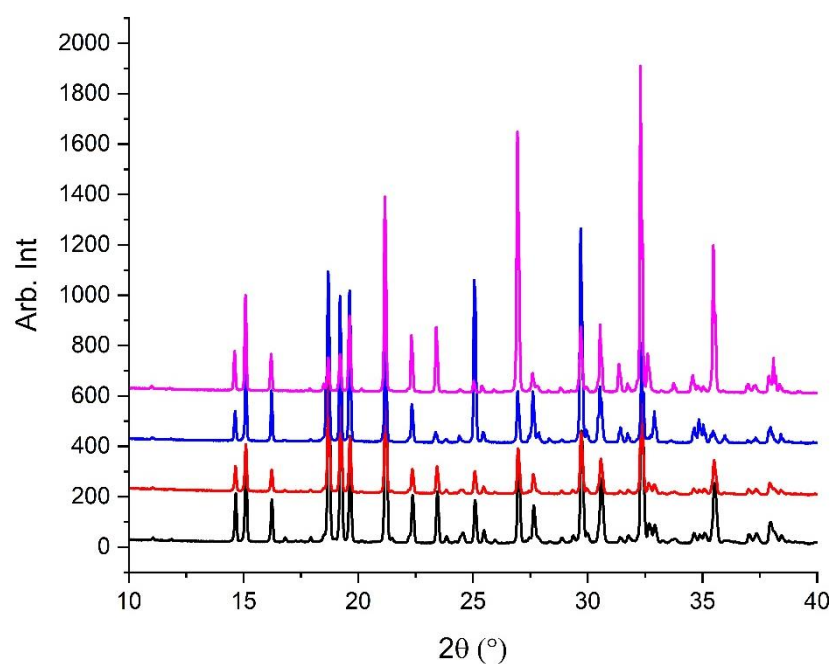


Figure C5.10. X-ray diffraction data for glycolide from the original source with (black and red) and without (blue and purple) cryomilling. Cryomilling has induced some conversion to the hydrolysis product (2-(2-hydroxyacetoxy)acetic acid; HAA) (peak at 17°).

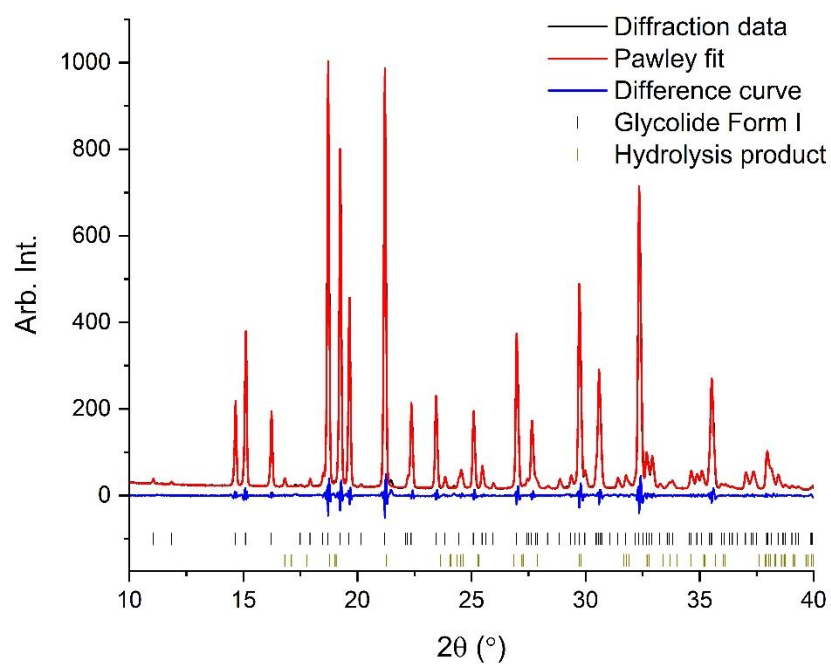


Figure C5.11. Pawley fit of the cryomilled sample using glycolide form I as a reference (black tick marks) and the hydrolysis product (2-(2-hydroxyacetoxy)acetic acid; HAA) as a reference (green tick marks).

Table C5.3. Details of the tablets prepared for this study. 20 tablets were selected to be examined using XRD-CT.

Tablet name	Tablet weight (mg)	Actual compaction pressure (MPa)	Was the tablet selected for study?
50_1	52.7	53	-
50_2	52.7	51	-
50_3	52.6	50	Yes
50_4	53.7	55	-
50_5	51.8	47	-
50_6	52.9	52	
100_1	59.7	92	-
100_2	61.6	104	Yes
100_3	61.5	105	-
100_4	61.2	101	-
100_5	60.9	104	-
100_6	61.4	106	-
130_1	62.2	113	-
130_2	63.9	129	-
130_3	64.9	137	Yes
130_4	64.4	137	-
130_5	64.2	135	-
130_6	64.1	135	-
150_1	65.0	167	-
150_2	62.9	121	-
150_3	64.8	146	-
150_4	64.4	137	-
150_5	65.2	149	-

150_6	65.2	146	-
150_7	65.9	157	-
150_8	66.3	164	-
150_9	65.8	149	-
170_1	63.2	144	-
170_2	65.0	170	-
170_3	64.7	164	-
170_4	64.0	153	Yes
170_5	66.8	198	-
170_6	64.7	166	Yes
200_1	66.1	195	-
200_2	65.8	189	-
200_3	66.6	200	Yes
200_4	67.4	220	Yes
200_5	66.9	211	Yes
200_6	66.7	203	Yes
300_1	70.7	317	Yes
300_2	69.8	296	-
300_3	70.9	329	Yes
300_4	69.6	283	Yes
300_5	71.4	352	Yes
300_6	70.2	299	Yes
400_1	71.8	364	Yes
400_2	75.5	548	Yes
400_3	73.5	440	Yes
400_4	75.1	520	Yes

400_5	73.8	454	-
400_6	73.1	423	-
400_7	72.6	307	Yes
400_8	75.1	392	Yes

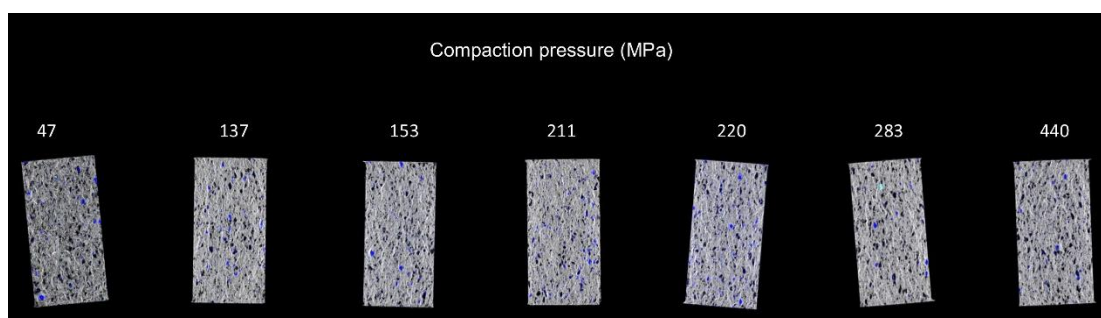


Figure C5.12. XRD-CT images of tablets compacted at different pressures. MCC is depicted as white and the unknown phases (2-(2-hydroxyacetoxy)acetic acid) is blue.

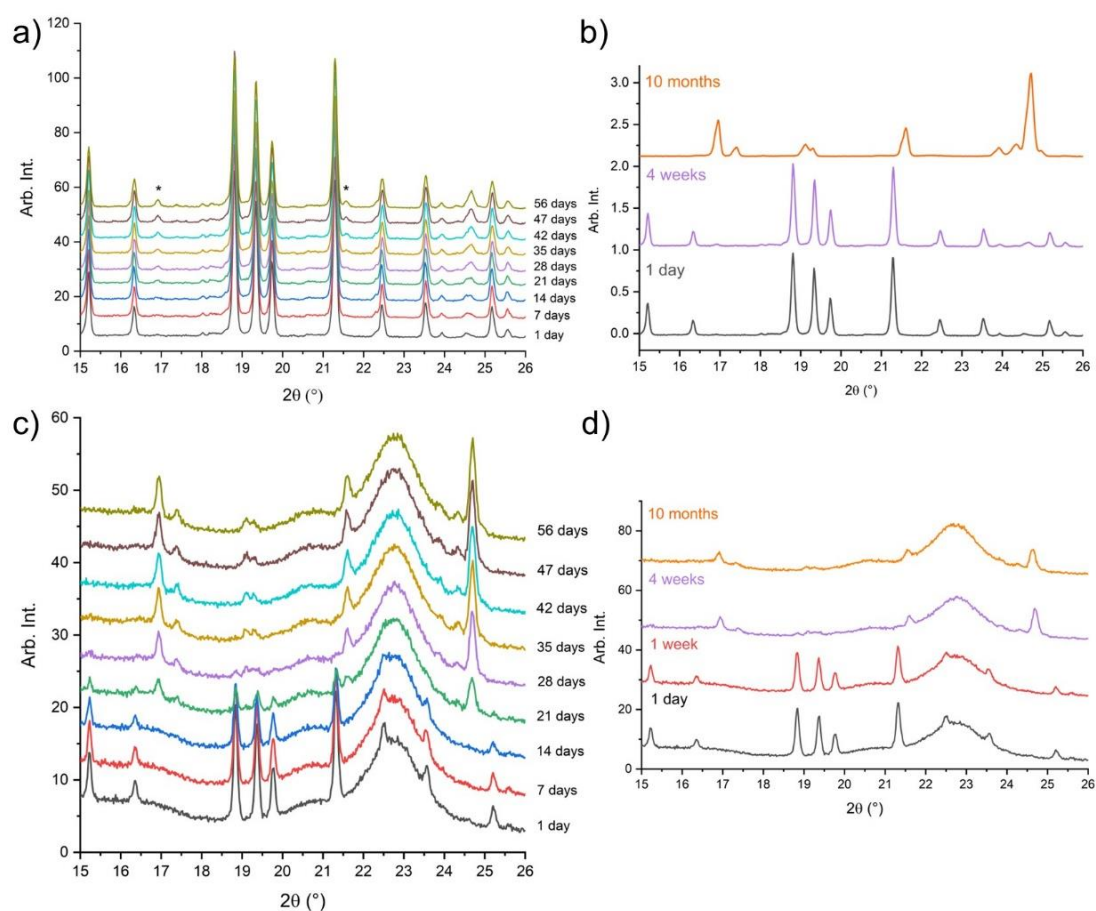


Figure C5.13. Diffraction patterns for pure glycolide (a & b) and the blended mixture used in the tableting part of the study (c & d). After 21 days there is significant transformation in the blended mixture with complete change in 35 days. This contrasts with the pure material where the transformation begins after 14 days but remains incomplete over 47 days. After approx. 10 months the transformation is complete in the pure substance.

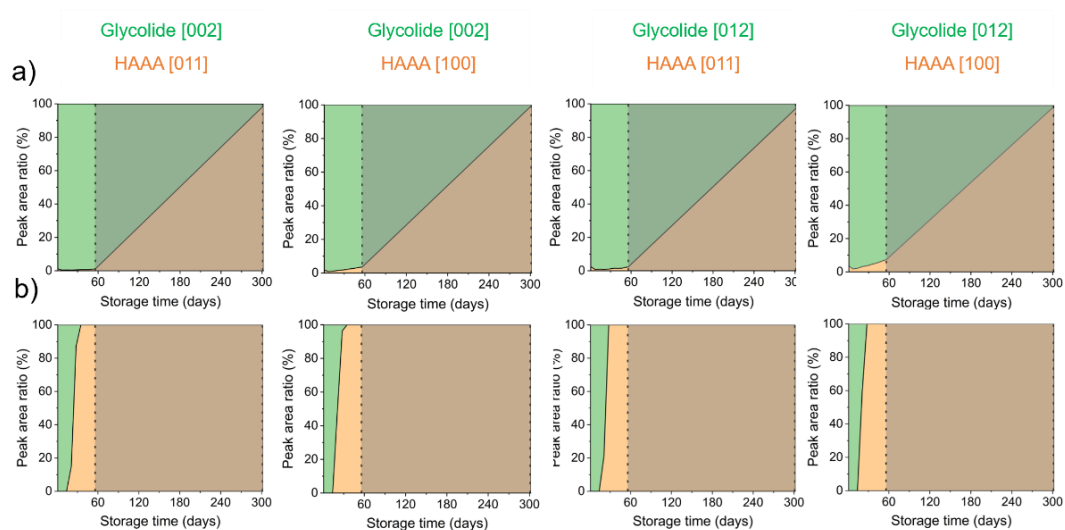


Figure C5.14. Conversion of glycolide Form I to 2-(2-hydroxyacetoxy)acetic acid in pure (a) and blended (b) mixtures upon storage. The change in the area under two characteristic peaks from the XRPD patterns of each compound is monitored over time. There is a faster conversion observed in the blended mixture over the pure compound. Glycolide peak areas are shown in green, 2-(2-hydroxyacetoxy)acetic acid peak areas are shown in orange. The shadowed area indicates that no data was collected between 2 and 10 months. The [002]/[100] pair is reproduced in Figure 5.8(c,e).

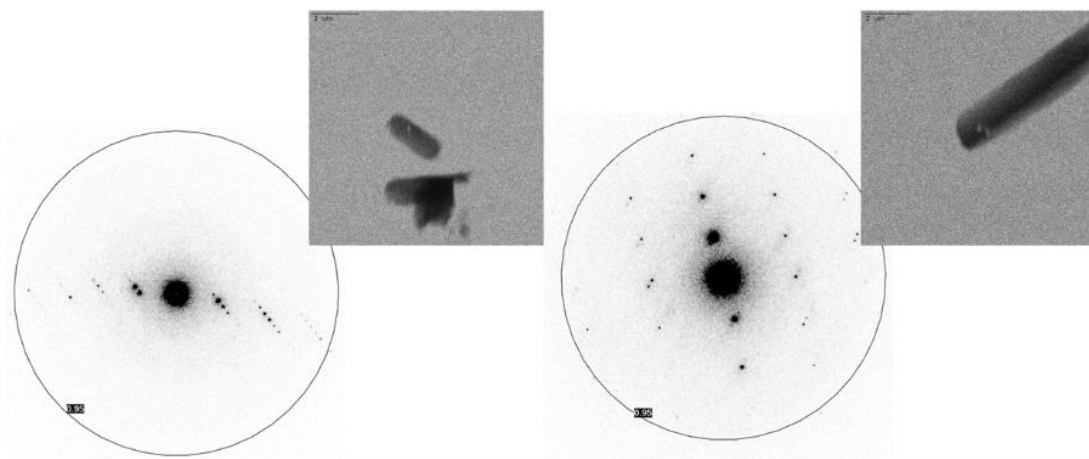


Figure C5.15. Exemplary diffraction and STEM (field of view 10 μm) images for crystal 1 (left) and crystal 3 (right).

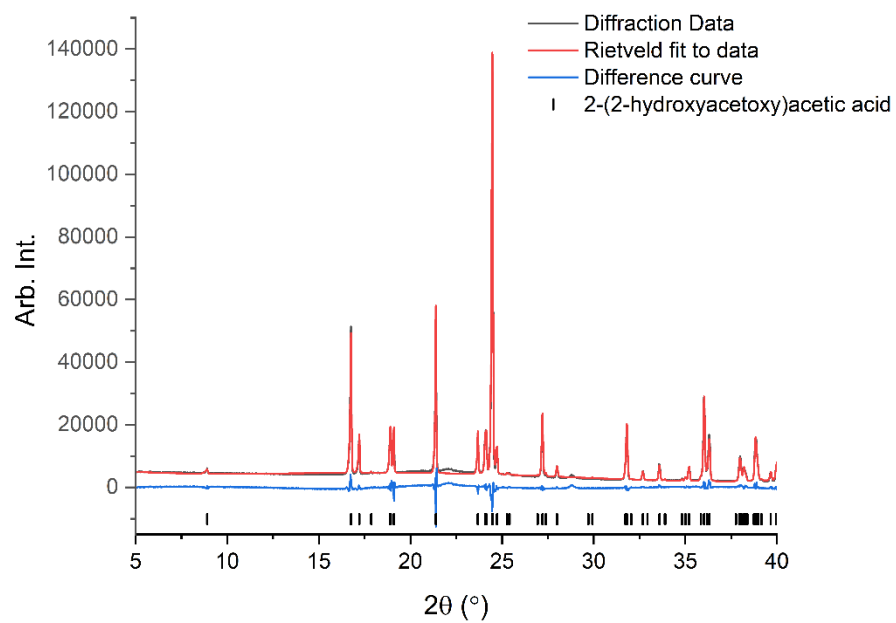


Figure C5.16. Rietveld fit of 2-(2-hydroxyacetoxy)acetic acid using the coordinates from the crystal structure determination from electron diffraction.

Table C5.4. The crystallographic parameters for 2-(2-hydroxyacetoxy)acetic acid as determined by electron diffraction and Rietveld analysis of the powder.

	2-(2-hydroxyacetoxy) acetic acid	2-(2-hydroxyacetoxy) acetic acid
Crystal data	Electron diffraction	Rietveld fit
Chemical formula	C ₄ H ₆ O ₅	C ₄ H ₆ O ₅
M_r	134.09	134.09
Crystal system, space group	Monoclinic, $P2_1/c$	Monoclinic, $P2_1/c$
Temperature (K)	298	298
a, b, c (Å)	5.28 (5), 5.35 (5), 19.92 (19)	5.28703 (18), 5.33231 (16), 19.8459 (7)
β (°)	90.900(13)	90.612(2)
V (Å ³)	562 (9)	559.46(3)
Z	4	4
Radiation type	$\lambda = 0.02851$ Å (electrons)	$\lambda = 1.54043$ Å (X-rays)
μ (mm ⁻¹)	0.000	1.349388
Crystal size (mm)	××	Powder
Data collection		
Diffractometer	ELDICO <i>ED-1</i>	
Absorption correction	Multi-scan <i>SADABS2016/2</i> (Bruker, 2016/2) was used for absorption correction. $wR^2(\text{int})$ was 0.1704 before and 0.1232 after correction. The Ratio of minimum to maximum transmission is 0.2545. The 1/2 correction factor is not present.	
$T_{\min}, T_{\max}$	0.189, 0.743	

No. of measured, independent and observed [$I > 2s(I)$] reflections	4316, 847, 693	
R_{int}	0.099	
$(\sin \theta/\lambda)_{\text{max}}$ ($\text{\AA}^{-1}$)	0.602	
Refinement		
$R[F^2 > 2s(F^2)]$, $wR(F^2)$, S	0.159, 0.422, 1.73	$R_p = 0.062$, $R_{wp} = 0.080$, $R_{\text{exp}} = 0.015$, $\chi^2 = 28.933$
No. of reflections	847	
No. of parameters	89	
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement	
$\Delta\rho_{\text{max}}$, $\Delta\rho_{\text{min}}$ ($\text{e \AA}^{-3}$)	0.18, -0.22	

Computer programs: Eldix⁴⁴, SAINT⁴⁶, SHELXT⁴⁸, SHELXL⁴⁹, Olex2 1.5⁸⁰.

Chapter 6 - Conclusions and Future Work

6.1. Conclusions

The three research chapters covered in this thesis built on the overall aim to advance the understanding of the structural response of pharmaceutical solids to applied pressure, from the molecular to the macroscopic scale, with relevance to the pharmaceutical tableting process.

Chapter 3 conducted a high-pressure crystallographic study of a chiral and racemic model system (levofloxacin and ofloxacin) across different pressure conditions (hydrostatic and non-hydrostatic) and different magnitudes (spanning tableting-relevant to high pressures). The three forms studied (ofloxacin, γ -levofloxacin and levofloxacin hemihydrate) were shown to be soft and highly compressible van der Waals solids, with their low bulk moduli reflecting the dominance of dispersive π -stacking interactions. PIXEL calculations supported this, with ofloxacin found to be more stable than γ -levofloxacin owing to the greater dispersive contribution of its stacking interactions. In each case, the most compressible direction was perpendicular to the molecular stacking. All three forms compressed smoothly through the tableting range, with evidence of pressure-induced phase transitions only at higher pressures (γ -levofloxacin at *ca.* 1.14 GPa, ofloxacin above 4.65 GPa and levofloxacin hemihydrate near 5.1 GPa). Despite the close relationship among the three structures, they exhibited different pressure-induced phase behaviour dependent on the pressure-transmitting medium and particle size, as identified by X-ray diffraction and, in the case of levofloxacin hemihydrate, confirmed by Raman spectroscopy. The phase transition in ofloxacin was shown to be driven by volume decrease rather than by shear, as it

occurred at a similar pressure under both hydrostatic and non-hydrostatic conditions. Collectively, these findings illustrate how stereochemistry, hydration and external conditions interact to govern compressibility and phase stability, which are directly relevant to solid-form selection and the structural robustness of these materials during pharmaceutical processing steps.

The conventional crystallographic analyses used in Chapter 3, such as the principal axes of strain, describe how the unit cell responds to pressure but capture molecular displacement only indirectly and within the constraints of crystallographic symmetry, which can overlook subtle rearrangements. Chapter 4 addressed this limitation and developed a new method to provide direct insight into molecular displacement from variable-pressure diffraction data, along with a Python-based tool (MolDis) to implement it. Molecular displacements were visualised and evaluated for five structures. The method was first validated against paracetamol Form I and Form II, reproducing the known structural responses and thereby confirming its reliability, while also providing additional insight. It was then applied to three structurally related acetanilides whose high-pressure behaviour had not previously been reported: the monoclinic form of 4-methylacetanilide, 4-chloroacetanilide and metacetamol Form I. Across the systems studied, molecular displacements showed that corrugated chains and layers tended to flatten, potential slip planes without bridging hydrogen bonds and a wide interlayer separation appeared to facilitate directional displacement normal to the plane, and hydrogen-bonded contacts continued to approach one another despite increasing energetic destabilisation in the monoclinic form of 4-methylacetanilide, 4-chloroacetanilide and metacetamol Form I. Used alongside interaction energy, void

and slip plane calculations, MolDis complemented conventional analyses and provided molecular-level insights beyond them.

Chapter 5 moved from the molecular to the macroscopic scale and applied X-ray Diffraction Computed Tomography (XRD-CT) to intact pharmaceutical tablets for the first time, to the best of our knowledge. Tablets containing glycolide were studied because Form I's structure undergoes a reconstructive phase transformation to Form II at *ca.* 150 MPa, which is recoverable upon decompression. A non-destructive molecular-level spatial and temporal investigation of glycolide's polymorphic transformation within tablets revealed that the phase transformation was not uniform across the tablet. Form I transformed in the centre of the tablets while remaining unchanged at the edges, and this change in the distribution of Form I became evident approximately 50 MPa above the hydrostatic transition pressure. The presence of Form II became significant above *ca.* 283 MPa, with the formulation's high microcrystalline cellulose content (80 wt%) raising the transformation pressure relative to the pure material. The data also captured the annealing of particles into larger crystallites and their subsequent fragmentation at higher compaction pressures. A follow-up study one month later unexpectedly revealed a hydrolysis reaction, in which glycolide converted to a ring-opening product, 2-(2-hydroxyacetoxy)acetic acid; the crystal structure of this previously unreported form was determined by electron diffraction, enabling structure elucidation directly from a nanocrystalline material (i.e., crystallites within a powder sample) that would otherwise be inaccessible by conventional single crystal X-ray diffraction without extensive recrystallisation experiments to grow suitably large crystals. This provides a clear example of the advances offered by this technique and highlights its growing importance for characterising challenging pharmaceutical

samples. The study established XRD-CT as a powerful, non-destructive technique to spatially resolve solid-form changes in low-symmetry organic compounds, representing an advancement in how pharmaceutical tablets can be studied.

Overall, although each study stands on its own, together they follow the structural response to pressure across scales: from how molecular structures and intermolecular interactions respond to pressure, to how individual molecules' responses can be made explicit and visualised, to where and to what extent structural changes occur inside a formulated tablet. This combined perspective provides a more comprehensive understanding, improving our ability to anticipate and control solid form change during manufacturing.

6.2. Future Work

Specific avenues for future work based on the methodology developed in Chapter 4 are proposed there. This section builds on those and proposes additional directions that cut across the work carried out in this thesis, with the goal of advancing the understanding of the structural response of pharmaceutical solids to applied pressure, particularly in a tableting context.

A natural progression from this work would be to apply the experimental approaches used in this thesis to the same systems, connecting how and why a material transforms under pressure with the extent to which it does so in a pharmaceutical tablet. Glycolide would provide an excellent starting point, since it has already been tested across length scales and high-pressure/tableting environments. Examining how glycolide molecules translate before Form I transforms to Form II using MolDis would offer insight into the structural changes that precede this transition. Realising this would depend on collecting data at sufficient pressure points before the transition occurs, and on the translations across this pressure range being large enough relative to the atomic displacement parameters and related centroid uncertainties to map displacement reliably.

The compressibility and phase behaviour of ofloxacin, γ -levofloxacin and levofloxacin hemihydrate established in Chapter 3 could be related to their mechanical properties by computing their elastic stiffness tensors or measuring hardness and indentation modulus on specific crystal faces using nanoindentation, connecting crystallographic observations to the mechanical behaviour that influences how a material compresses to form a tablet.

Building on the role of slip planes, relating known or suspected slippage behaviour to the response under hydrostatic conditions of the same systems would help establish whether slip systems can be inferred experimentally from molecular displacement. A specific experiment would be to determine whether the potential (002) slip plane in the monoclinic form of 4-methylacetanilide confers the enhanced plasticity and tableting performance observed in paracetamol Form II, providing a direct link between hydrostatic molecular translation and macroscopic deformation.

More broadly, collecting high-pressure crystallographic datasets across a wider range of systems would build a large database suitable for data mining. This would extend the existing Cambridge Structural Database (CSD) data at the intersection of the CSD Drug Subset and High Pressure Subset. Building an extensive database would require an efficient data collection process. The timeline for collecting high-pressure X-ray diffraction datasets could be shortened by loading a diamond anvil cell (DAC) with multiple crystals to collect data from different samples under the same sample loading, using a gasket with multiple smaller sample chambers (i.e., holes) to ensure that changes to the samples are not impacted by the neighbouring samples (e.g., partial dissolution of a crystal in the pressure-transmitting media). A pressure marker would be introduced in each sample chamber for accurate pressure readings.

Data could be collected either in-house or at a synchrotron to take advantage of shorter collection times. The collection of high-pressure data could be automated by using a membrane-driven DAC (mDAC) under closed-loop control, in which the membrane pressure is adjusted until the ruby R1 shift indicates the target pressure has been reached, at which point data are collected before the system moves on to the next

pressure point. Throughput could be further increased by automatically cycling through a set of loaded mDACs.

Analysing additional data using MolDis, as well as conventional analyses, would offer an opportunity to identify trends in pressure-driven responses from structural features and intermolecular interactions, building toward a predictive understanding of pressure-induced phase behaviour. If these analyses were to be automated, the database and related quantitative output from the analyses could be used to train machine learning (ML) models that learn how structural features and intermolecular interactions relate to compressibility and the onset of pressure-induced transitions, uncovering trends across more systems than case-by-case analysis would allow.

Such datasets would be most powerful if built around systems whose mechanical properties or slip systems have already been characterised, so that these structural descriptors and interaction energies could be correlated directly with their known mechanical behaviour. The elasticity, plasticity and breakage of pharmaceutical materials could be understood in terms of principal axes of strain, bulk modulus, slip planes, void space and surface attachment energies. Establishing these structure-property relationships would not only provide a mechanistic description of mechanical behaviour and help explain why and how particles deform and break upon processing, but also establish relative rankings to assess polymorphs and solid forms. Trained on a sufficiently large high-pressure database, an ML model of these relationships could be applied to screen the wider CSD and select promising systems to study, flagging structures with particular potential slip planes or surface attachment energies.

Having established in this thesis the first reported application of XRD-CT to formulated pharmaceutical tablets, an exciting next step is to extend this technique

across a broader range of APIs and formulations. The impact of a formulation on an API's polymorphic form under tableting conditions could be investigated by resolving the presence and extent of phase transformations under varying drug loadings and excipient combinations.

Beyond examining changes retained upon tablet relaxation, developing a beamline XRD-CT set-up for *in situ* data collection from a tablet press would allow the molecular (i.e., crystal structure) and microstructure changes within a tablet to be followed as they develop under load, spatially resolving the fragmentation and annealing of individual components, and capturing phase transitions that revert to the ambient phase upon decompression and tablet relaxation. This would complement the particle and tablet microstructure characterisation obtained from X-ray tomography. The same set-up would also help isolate the role of shear, which could be probed further through high-pressure X-ray powder diffraction collected without a pressure-transmitting medium.