4.5.2. X-ray fibre diffraction analysis

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4.5.2.1. Introduction

X-ray fibre diffraction analysis is a collection of crystallographic techniques that are used to determine molecular and crystal structures of molecules, or molecular assemblies, that form specimens (often fibres) in which the molecules, assemblies or crystallites are approximately parallel but not otherwise ordered (Arnott, 1980; French & Gardner, 1980; Hall, 1984; Vibert, 1987; Millane, 1988; Atkins, 1989; Stubbs, 1999). These are usually long, slender molecules and they are often inherently flexible, which usually precludes the formation of regular three-dimensional crystals suitable for conventional crystallographic analysis. X-ray fibre diffraction therefore provides a route for structure determination for certain kinds of specimens that cannot be crystallized. Although it may be possible to crystallize small *fragments* or *subunits* of these molecules, and determine the crystal structures of these, X-ray fibre diffraction provides a means for studying the intact, and often the biologically or functionally active, system. Fibre diffraction has played an important role in the determination of biopolymers such as polynucleotides, polysaccharides (both linear and branched), polypeptides and a wide variety of synthetic polymers (such as polyesters), as well as larger assemblies including rod-like helical viruses, bacteriophages, microtubules and muscle fibres (Arnott, 1980; Arnott & Mitra, 1984; Millane, 1990*c*; Squire & Vibert, 1987).

Specimens appropriate for fibre diffraction analysis exhibit rotational disorder (of the molecules, aggregates or crystallites) about a preferred axis, resulting in cylindrical averaging of the diffracted intensity in reciprocal space. Therefore, fibre diffraction analysis can be thought of as 'structure determination from cylindrically averaged diffraction intensities' (Millane, 1993). In a powder specimen the crystallites are completely (spherically) disordered, so that structure determination by fibre diffraction can be considered to be intermediate between structure determination from single crystals and from powders.

This section is a review of the theory and techniques of structure determination by X-ray fibre diffraction analysis. It includes descriptions of fibre specimens, the theory of diffraction by these specimens, intensity data collection and processing, and the variety of structure determination methods used for the various kinds of specimens studied by fibre diffraction. It does not include descriptions of specimen preparation (those can be found in the references given for specific systems), or of applications of X-ray diffraction to determining polymer morphology (*e.g.* particle or void sizes and shapes, texture, domain structure *etc.*).

4.5.2.2. Fibre specimens

A wide variety of kinds of fibre specimen exist. All exhibit preferred orientation; the variety results from variability in the degree of order (crystallinity) in the lateral plane (the plane perpendicular to the axis of preferred orientation). This leads to categorization of three kinds of fibre specimen: *noncrystalline fibres*, in which there is no order in the lateral plane; *polycrystalline fibres*, in which there is near-perfect crystallinity in the lateral plane; and *disordered fibres*, in which there is disorder either within the molecules or in their crystalline packing (or both). The kind of fibre specimen affects the kind of diffraction pattern obtained, the relationships between the molecular and crystal structures and the diffraction data, methods of data collection, and methods of structure determination.

Noncrystalline fibres are made up of a collection of molecules that are *oriented*. This means that there is a common axis in each molecule (referred to here as the *molecular axis*), the axes being parallel in the specimen. The direction of preferred orientation is called the *fibre axis*. The molecule itself is usually considered to be a rigid body. There is no other ordering within the specimen. The molecules are therefore randomly positioned in the lateral plane and are randomly rotated about their molecular axes. Furthermore, if the molecule does not have a twofold rotation axis normal to the molecular axis, then the molecular axis has a *direction* associated with it, and the molecular axes are oriented randomly parallel or antiparallel to each other. This is often called *directional disorder*, or the molecules are said to be oriented *randomly up and down*. The average length of the ordered molecular segments in a noncrystalline fibre is referred to as the *coherence length*.

Polycrystalline fibres are characterized by molecular segments packing together to form well ordered microcrystallites within the specimen. The crystallites effectively take the place of the molecules in a noncrystalline specimen as described above. The crystallites are oriented, and since the axis within each crystallite that is aligned parallel to those in other crystallites usually corresponds to the long axes of the constituent molecules, it is also referred to here as the molecular axis. The crystallites are randomly positioned in the lateral plane, randomly rotated about the molecular axis, and randomly oriented up or down. The size of the crystalline domains can be characterized by their average dimensions in the directions of the **a**, **b** and **c** unit-cell vectors. However, because of the rotational disorder of the crystallites, any differences between crystallite dimensions in different directions normal to the fibre axis tend to be smeared out in the diffraction pattern, and the crystallite size is usefully characterized by the average dimensions of the crystallites normal and parallel to the fibre axis.

The molecules or crystallites in a fibre specimen are not perfectly oriented, and the variation in inclinations of the molecular axes to the fibre axis is referred to as *disorientation*. Assuming that the orientation is axisymmetric, then it can be described by an *orientation density function* $\Omega(\alpha)$ such that $\Omega(\alpha)\,\mathrm{d}\omega$ is the fraction of molecules in an element of solid angle $\mathrm{d}\omega$ inclined at an angle α to the fibre axis. The exact form of $\Omega(\alpha)$ is generally not known for any particular fibre and it is often sufficient to assume a Gaussian orientation density function, so that

$$\Omega(\alpha) = \frac{1}{2\pi\alpha_0^2}\exp\left(-\frac{\alpha^2}{2\alpha_0^2}\right), \qquad (4.5.2.1)$$

where α_0 is a measure of the degree of disorientation.

Fibre specimens often exhibit various kinds of disorder. The disorder may be within the molecules or in their packing. Disorder affects the relationship between the molecular and crystal structure and the diffracted intensities. Disorder within the molecules may result from a degree of randomness in the chemical sequence of the molecule or from variability in the interactions between the units that make up the molecule. Such molecules may (at least in principle) form noncrystalline, polycrystalline or partially crystalline (described below) fibres. Disordered packing of molecules within crystallites can result from a variety of ways in which the molecules can interact with each other. Fibre specimens made up of disordered crystallites are referred to here as partially crystalline fibres.

4.5.2.3. Diffraction by helical structures

Molecules or assemblies studied by fibre diffraction are usually made up of a large number of identical, or nearly identical, residues, or subunits, that in an oriented specimen are distributed along an axis; this leads naturally to helical symmetry. Since a periodic structure with no helix symmetry can be treated as a onefold helix, the assumption of helix symmetry is not restrictive.