$$R = \frac{\sum_m N_m R_m S_m}{\sum_m N_m S_m}, \qquad (4.5.2.77)$$

where the sums are over the values of m on the diffraction pattern, N_m is the number of data that have m components, R_m is given by equation (4.5.2.75) and S_m is given by

$$S_m = \frac{\Gamma\big((m/2) + (1/2)\big)}{\Gamma(m/2)}, \qquad (4.5.2.78)$$

where $\Gamma(\cdot)$ is the gamma function. The quantities on the right-hand side of equation (4.5.2.77) are easily determined for a particular data set. The largest likely R factor decreases (since m increases) with increasing resolution of the data, increasing diameter of the molecule and decreasing order u of the helix symmetry. For example, for TMV at 5 Å resolution the largest likely R factor is 0.37, and at 3 Å resolution it is 0.31, whereas for a tenfold nucleic acid structure at 3 Å resolution it is 0.40 (Millane, 1989*b*, 1992*b*). This underlines the importance of comparing R factors obtained in a fibre diffraction analysis with the largest likely R factor; an R factor of 0.25 that may indicate a good protein structure may, or may not, indicate a well determined fibre structure.

Using approximations for R_m, S_m and m allows the following approximation for the largest likely R factor for a noncrystalline fibre to be derived (Millane, 1992*b*):

$$R \simeq 0.261(ud_{\max}/r_{\max})^{1/2}, \qquad (4.5.2.79)$$

where $d_{\max}$ is the resolution of the data. The approximation (4.5.2.79) is generally not good enough for calculating accurate largest likely R factors, but it does show the general behaviour with helix symmetry, molecular diameter and diffraction-data resolution. Other approximations to largest likely R factors have been derived that are quite accurate and also include the effect of a minimum resolution for the data (Millane, 1992*b*).

Largest likely R factors in fibre diffraction studies are typically between about 0.3 and 0.5, depending on the particular structure (Millane, 1989*b*, 1992*b*; Millane & Stubbs, 1992). Although the largest likely R factor does not give a quantitative assessment of the significance of an R factor obtained in a particular structure determination, it can be used as a guide to the significance. R factors obtained for well determined protein structures are typically between about one-third and one-half of the corresponding largest likely R factor, depending on the resolution. It is therefore reasonable to expect the R factor for a well determined fibre structure to be between one-third and one-half of the largest likely R factor calculated for the structure. R factors should, therefore, generally be less than 0.15 to 0.25, depending on the particular structure and the resolution as illustrated by the examples presented in Millane & Stubbs (1992).

The free R factor (Brünger, 1997) has become popular in single-crystal crystallography as a tool for validation of refinements. The free R factor is more difficult to implement (but is probably even more important) in fibre diffraction studies because of the smaller data sets, but has been used to advantage in recent studies (Hudson *et al.*, 1997; Welsh *et al.*, 1998, 2000).

4.5.3. Electron crystallography of polymers

(D. L. DORSET)

4.5.3.1. *Is polymer electron crystallography possible?*

As a crystallographic tool, the electron microscope has also made an important impact in polymer science. Historically, single-crystal electron diffraction information has been very useful for the interpretation of cylindrically averaged fibre X-ray patterns (Atkins, 1989), particularly when there is an extensive overlap of diffracted intensities. An electron diffraction pattern aids indexing of the fibre pattern and facilitates measurement of unit-cell constants, and the observation of undistorted plane-group symmetry similarly places important constraints on the identification of the space group (Geil, 1963; Wunderlich, 1973).

The concept of using electron diffraction intensities by themselves for the quantitative determination of crystal structures of polymers or other organics often has been met with scepticism (Lipson & Cochran, 1966). Difficulties experienced in the quantitative interpretation of images and diffraction intensities from 'hard' materials composed of heavy atoms (Hirsch *et al.*, 1965; Cowley, 1981), for example, has adversely affected the outlook for polymer structure analysis, irrespective of whether these reservations are important or not for 'soft' materials comprising light atoms. Despite the still commonly held opinion that no new crystal structures will be determined that are solely based on data collected in the electron microscope, it can be shown that this extremely pessimistic outlook is unwarranted. With proper control of crystallization (*i.e.* crystal thickness) and data collection, the electron microscope can be used quite productively for the direct determination of macromolecular structures at atomic resolution, not only to verify some of the previous findings of fibre X-ray diffraction analysis, but, more importantly, to determine new structures, even of crystalline forms that cannot be studied conveniently by X-rays as drawn fibres (Dorset, 1995*b*). The potential advantages of electron crystallography are therefore clear. The great advantage in scattering cross section of matter for electrons over X-rays permits much smaller samples to be examined by electron diffraction as single-crystalline preparations (Vainshtein, 1964). (Typical dimensions are given below.)

Electron crystallography can be defined as the quantitative use of electron micrographs and electron diffraction intensities for the determination of crystal structures. In the electron microscope, an electron beam illuminates a semitransparent object and the microscope objective lens produces an enlarged representation of the object as an image. If the specimen is thin enough and/or the electron energy is high enough, the weak-phase-object or 'kinematical' approximation is valid (Cowley, 1981), see Chapter 2.5. That is to say, there is an approximate one-to-one mapping of density points between the object mass distribution and the image, within the resolution limits of the instrument (as set by the objective lens aberrations and electron wavelength). The spatial relationships between diffraction and image planes of an electron microscope objective lens are reciprocal and related by Fourier transform operations (Cowley, 1988). While it is easy to transform from the image to the diffraction pattern, the reverse Fourier transform of the diffraction pattern to a high-resolution image requires solution of the famous crystallographic phase problem (as discussed for electron diffraction in Section 2.5.7).

Certainly, in electron diffraction studies, one must still be cognizant of the limitations imposed by the underlying scattering theory. An approximate 'quasi-kinematical' data set is often sufficient for the analysis (Dorset, 1995*a*). However (Dorset, 1995*b*), there are other important perturbations to diffraction intensities which should be minimized. For example, the effects of radiation damage while recording a high-resolution image are minimized by so-called 'low-dose' procedures (Tsuji, 1989).

4.5.3.2. *Crystallization and data collection*

The success of electron crystallographic determinations relies on the possibility of collecting data from *thin* single microcrystals. These can be grown by several methods, including self-seeding, epitaxic orientation, *in situ* polymerization on a substrate, in a Langmuir–Blodgett layer, *in situ* polymerization within a thin layer

references