determination (Raghavan, 1961; Moon, 1961) as the anomalous-scattering effects in γ-ray scattering could be very large; the wavelength is also easily tunable. However, the intensity obtainable for γ-rays is several orders lower than that obtainable from X-ray and neutron sources, and hence γ-ray anomalous scattering is of hardly any practical value in structural analysis.

2.4.5.1. *Neutron anomalous scattering*

Apart from the limitations introduced by experimental factors, such as the need for large crystals and the comparatively low flux of neutron beams, there are two fundamental reasons why neutrons are less suitable than X-rays for the *ab initio* determination of crystal structures. First, the neutron scattering lengths of different nuclei have comparable magnitudes whereas the atomic form factors for X-rays vary by two orders of magnitude. Therefore, Patterson techniques and the related heavy-atom method are much less suitable for use with neutron diffraction data than with X-ray data. Secondly, neutron scattering lengths could be positive or negative and hence, in general, the positivity criterion (Karle & Hauptman, 1950) or the squarability criterion (Sayre, 1952) does not hold good for nuclear density. Therefore, the direct methods based on these criteria are not strictly applicable to structure analysis using neutron data, although it has been demonstrated that these methods could be successfully used in favourable situations in neutron crystallography (Sikka, 1969). The anomalous-scattering method is, however, in principle more powerful in the neutron case than in the X-ray case for *ab initio* structure determination.

Thermal neutrons are scattered anomalously at appropriate wavelengths by several nuclei. In a manner analogous to (2.4.3.1), the neutron scattering length of these nuclei can be written as

$$b_0 + b' + ib'' = b + ib''. \qquad (2.4.5.1)$$

The correction terms b' and b'' are strongly wavelength-dependent. In favourable cases, b'/b_0 and b''/b_0 can be of the order of 10 whereas they are small fractions in X-ray anomalous scattering. In view of this pronounced anomalous effect in neutron scattering, Ramaseshan (1966) suggested that it could be used for structure solution. Subsequently, Singh & Ramaseshan (1968) proposed a two-wavelength method for unique structure analysis using neutron diffraction. The first part of the method is the determination of the positions of the anomalous scatterers from the estimated values of F_Q. The method employed for estimating F_Q is analogous to that using (2.4.4.9) except that data collected at two appropriate wavelengths are used instead of those from two isomorphous crystals. The second stage of the two-wavelength method involves phase evaluation. Referring to Fig. 2.4.3.2 and in a manner analogous to (2.4.3.5), we have

$$\sin\psi_1 = \frac{F_{N1}^2(+) - F_{N1}^2(-)}{4F_{N1}F''_{Q1}}, \qquad (2.4.5.2)$$

where $\psi = \alpha_N - \alpha_Q$ and subscript 1 refers to data collected at wavelength $\lambda 1$. Singh and Ramaseshan showed that $\cos\psi_1$ can also be determined when data are available at wavelength $\lambda 1$ and $\lambda 2$. We may define

$$F_m^2 = [F_N^2(+) + F_N^2(-)]/2 \qquad (2.4.5.3)$$

and we have from (2.4.3.3), (2.4.3.4) and (2.4.5.3)

$$F_N = (F_m^2 - F''^2_Q)^{1/2}. \qquad (2.4.5.4)$$

Then,

$$\cos\psi_1 = \frac{F_{m1}^2 - F_{m2}^2 - [(b_1^2 + b''^2_1) - (b_2^2 + b''^2_2)]x^2}{2(b_1 - b_2)F_{N1}x} + \frac{F_{Q1}}{F_{N1}}, \qquad (2.4.5.5)$$

where x is the magnitude of the temperature-corrected geometrical part of $\mathbf{F}_Q$. ψ_1 and hence α_{N1} can be calculated using (2.4.5.2) and (2.4.5.5). α_{N2} can also be obtained in a similar manner.

During the decade that followed Ramaseshan's suggestion, neutron anomalous scattering was used to solve half a dozen crystal structures, employing the multiple-wavelength methods as well as the methods developed for structure determination using X-ray anomalous scattering (Koetzle & Hamilton, 1975; Sikka & Rajagopal, 1975; Flook *et al.*, 1977). It has also been demonstrated that measurable Bijvoet differences could be obtained, in favourable situations, in neutron diffraction patterns from protein crystals (Schoenborn, 1975). However, despite the early promise held by neutron anomalous scattering, the method has not been as successful as might have been hoped. In addition to the need for large crystals, the main problem with using this method appears to be the time and expense involved in data collection (Koetzle & Hamilton, 1975).

2.4.5.2. *Anomalous scattering of synchrotron radiation*

The most significant development in recent years in relation to anomalous scattering of X-rays has been the advent of synchrotron radiation (Helliwell, 1984). The advantage of using synchrotron radiation for making anomalous-scattering measurements essentially arises out of the tunability of the wavelength. Unlike the characteristic radiation from conventional X-ray sources, synchrotron radiation has a smooth spectrum and the wavelength to be used can be finely selected. Accurate measurements have shown that values in the neighbourhood of 30 electrons could be obtained in favourable cases for f' and f'' (Templeton, Templeton, Phillips & Hodgson, 1980; Templeton, Templeton & Phizackerley, 1980; Templeton *et al.*, 1982). Schemes for the optimization of the wavelengths to be used have also been suggested (Narayan & Ramaseshan, 1981). Interestingly, the anomalous differences obtainable using synchrotron radiation are comparable in magnitude to the isomorphous differences normally encountered in protein crystallography. Thus, the use of anomalous scattering at several wavelengths would obviously eliminate the need for employing many heavy-atom derivatives. The application of anomalous scattering of synchrotron radiation for macromolecular structure analysis began to yield encouraging results in the 1980s (Helliwell, 1985). Intensity measurements from macromolecular X-ray diffraction patterns using synchrotron radiation at first relied primarily upon oscillation photography (Arndt & Wonacott, 1977). This method is not particularly suitable for accurately evaluating anomalous differences. Much higher levels of accuracy began to be achieved with the use of position-sensitive detectors (Arndt, 1986). Anomalous scattering, in combination with such detectors, has developed into a major tool in macromolecular crystallography (see *IT* F, 2001).

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references