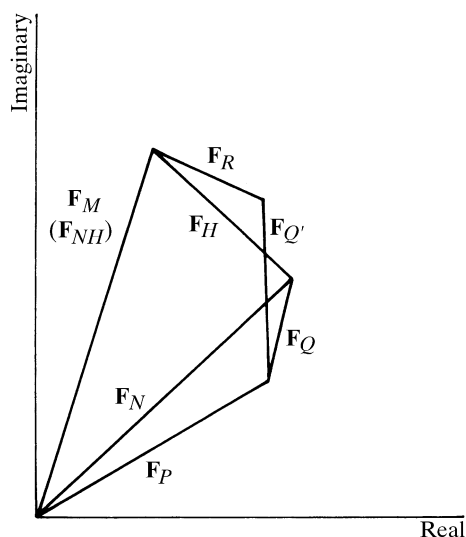2.4. ISOMORPHOUS REPLACEMENT AND ANOMALOUS SCATTERING

Fig. 2.4.2.1. Vector relationship between $\mathbf{F}_N$ and $\mathbf{F}_M$ ($\equiv \mathbf{F}_{NH}$).

replacement' will be used to cover both cases. Also, in as much as $\mathbf{F}_M$ is the vector sum of $\mathbf{F}_N$ and $\mathbf{F}_H$, $\mathbf{F}_M$ and $\mathbf{F}_{NH}$ will be used synonymously. Thus

$$\mathbf{F}_M \equiv \mathbf{F}_{NH} = \mathbf{F}_N + \mathbf{F}_H. \qquad (2.4.2.4)$$
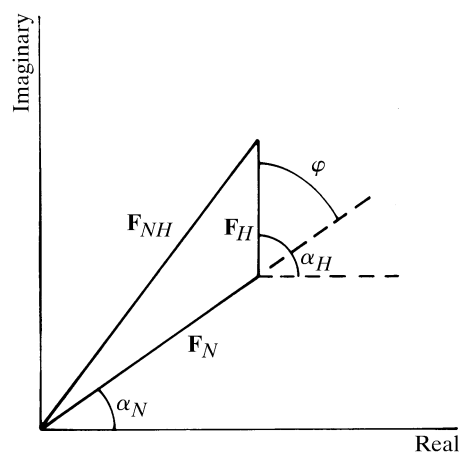
2.4.2.2. Single isomorphous replacement method

The number of replaceable (or 'added') atoms is usually small and they generally have high atomic numbers. Their positions are often determined by a Patterson synthesis of one type or another (see Chapter 2.3). It will therefore be assumed in the following discussion that $\mathbf{F}_H$ is known. Then it can be readily seen by referring to Fig. 2.4.2.2 that

$$\alpha_N = \alpha_H - \cos^{-1} \frac{F_{NH}^2 - F_N^2 - F_H^2}{2 F_N F_H} = \alpha_H \pm \varphi; \qquad (2.4.2.5)$$

when φ is derived from its cosine function, it could obviously be positive or negative. Hence, there are two possible solutions for α_N. These two solutions are distributed symmetrically about $\mathbf{F}_H$. One of these would correspond to the correct value of α_N. Therefore, in general, the phase angle cannot be unambiguously determined using a pair of isomorphous crystals.

The twofold ambiguity in phase angle vanishes when the structures are centrosymmetric. $\mathbf{F}_{NH}, \mathbf{F}_N$ and $\mathbf{F}_H$ are all real in

Fig. 2.4.2.2. Relationship between α_N, α_H and φ.

centric data and the corresponding phase angles are 0 or 180°. From (2.4.2.4)

$$F_{NH} \pm F_N = F_H. \qquad (2.4.2.6)$$

The sign of F_H is already known and the signs of F_{NH} and F_N can be readily determined from (2.4.2.6) (Robertson & Woodward, 1937).

When the data are acentric, the best one can do is to use both the possible phase angles simultaneously in a Fourier synthesis (Bokhoven *et al.*, 1951). This double-phased synthesis, which is equivalent to the isomorphous synthesis of Ramachandran & Raman (1959), contains the structure and its inverse when the replaceable atoms have a centrosymmetric distribution (Ramachandran & Srinivasan, 1970). When the distribution is noncentrosymmetric, however, the synthesis contains peaks corresponding to the structure and general background. Fourier syntheses computed using the single isomorphous replacement method of Blow & Rossmann (1961) and Kartha (1961) have the same properties. In this method, the phase angle is taken to be the average of the two possible solutions of α_N, which is always α_H or $\alpha_H + 180°$. Also, the Fourier coefficients are multiplied by cos φ, following arguments based on the Blow & Crick (1959) formulation of phase evaluation (see Section 2.4.4.4). Although Blow & Rossmann (1961) have shown that this method could yield interpretable protein Fourier maps, it is rarely used as such in protein crystallography as the Fourier maps computed using it usually have unacceptable background levels (Blundell & Johnson, 1976).

2.4.2.3. Multiple isomorphous replacement method

The ambiguity in α_N in a noncentrosymmetric crystal can be resolved only if at least two crystals isomorphous to it are available (Bokhoven *et al.*, 1951). We then have two equations of the type (2.4.2.5), namely,

$$\alpha_N = \alpha_{H1} \pm \varphi_1 \quad \text{and} \quad \alpha_N = \alpha_{H2} \pm \varphi_2, \qquad (2.4.2.7)$$

where subscripts 1 and 2 refer to isomorphous crystals 1 and 2, respectively. This is demonstrated graphically in Fig. 2.4.2.3 with the aid of the Harker (1956) construction. A circle is drawn with F_N as radius and the origin of the vector diagram as the centre. Two more circles are drawn with F_{NH1} and F_{NH2} as radii and the ends of vectors $-\mathbf{F}_{H1}$ and $-\mathbf{F}_{H2}$, respectively as centres. Each of these circles intersects the F_N circle at two points corresponding to the two possible solutions. One of the points of intersection is common and this point defines the correct value of α_N. With the assumption of perfect isomorphism and if errors are neglected, the phase circles corresponding to all the crystals would intersect at a common point if a number of isomorphous crystals were used for phase determination.

2.4.3. Anomalous-scattering method

2.4.3.1. Dispersion correction

Atomic scattering factors are normally calculated on the assumption that the binding energy of the electrons in an atom is negligible compared to the energy of the incident X-rays and the distribution of electrons is spherically symmetric. The transition frequencies within the atom are then negligibly small compared to the frequency of the radiation used and the scattering power of each electron in the atom is close to that of a free electron. When this assumption is valid, the atomic scattering factor is a real positive number and its value decreases as the scattering angle increases because of the finite size of the atom. When the binding energy of the electrons is appreciable, the atomic scattering factor at any given angle is given by

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Fig. 2.4.2.3. Harker construction when two heavy-atom derivatives are available.

(a)

(b)

Fig. 2.4.3.1. Variation of (a) f' and (b) f'' as a function of atomic number for Cu $K\alpha$ and Mo $K\alpha$ radiations. Adapted from Fig. 3 of Srinivasan (1972).
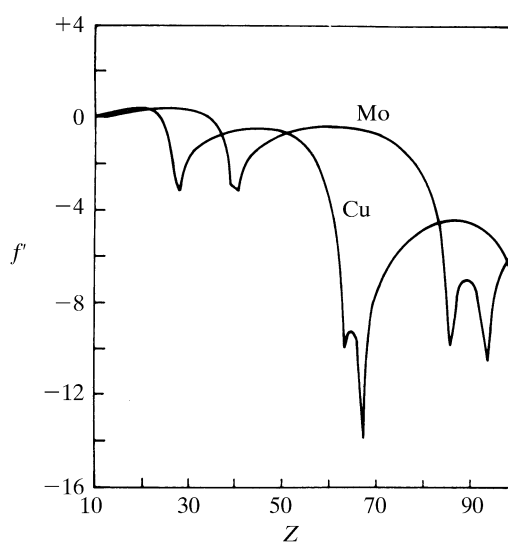
$$f_0 + f' + if'', \qquad (2.4.3.1)$$

where f_0 is a real positive number and corresponds to the atomic scattering factor for a spherically symmetric collection of free electrons in the atom. The second and third terms are, respectively, referred to as the real and the imaginary components of the 'dispersion correction' (*IT* IV, 1974). f' is usually negative whereas f'' is positive. For any given atom, f'' is obviously 90° ahead of the real part of the scattering factor given by
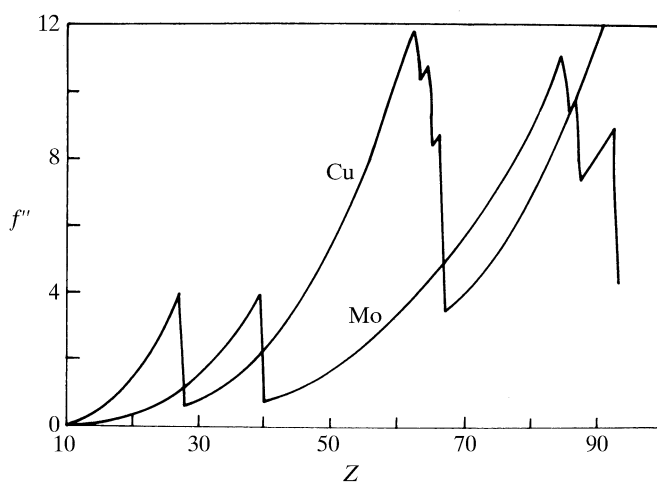
$$f = f_0 + f'. \qquad (2.4.3.2)$$

The variation of f' and f'' as a function of atomic number for two typical radiations is given in Fig. 2.4.3.1 (Srinivasan, 1972; Cromer, 1965). The dispersion effects are pronounced when an absorption edge of the atom concerned is in the neighbourhood of the wavelength of the incident radiation. Atoms with high atomic numbers have several absorption edges and the dispersion-correction terms in their scattering factors always have appreciable values. The values of f' and f'' do not vary appreciably with the angle of scattering as they are caused by core electrons confined to a very small volume around the nucleus. An atom is usually referred to as an anomalous scatterer if the dispersion-correction terms in its scattering factor have appreciable values. The effects on the structure factors or intensities of Bragg reflections resulting from dispersion corrections are referred to as anomalous-dispersion effects or anomalous-scattering effects.

2.4.3.2. *Violation of Friedel's law*

Consider a structure containing N atoms of which P are normal atoms and the remaining Q anomalous scatterers. Let $\mathbf{F}_P$ denote the contribution of the P atoms to the structure, and $\mathbf{F}_Q$ and $\mathbf{F}_Q''$ the real and imaginary components of the contribution of the Q atoms. The relation between the different contributions to a reflection $\mathbf{h}$ and its Friedel equivalent $-\mathbf{h}$ is illustrated in Fig. 2.4.3.2. For simplicity we assume here that all Q atoms are of the same type. The phase angle of $\mathbf{F}_Q''$ is then exactly 90° ahead of that of $\mathbf{F}_Q$. The structure factors of $\mathbf{h}$ and $-\mathbf{h}$ are denoted in the figure by $\mathbf{F}_N(+)$ and $\mathbf{F}_N(-)$, respectively. In the absence of anomalous scattering, or when the imaginary component of the dispersion correction is zero, the magnitudes of the two structure factors are equal and Friedel's law is obeyed; the phase angles have equal magnitudes, but opposite signs. As can be seen from Fig. 2.4.3.2, this is no longer true when

$\mathbf{F}_Q''$ has a nonzero value. Friedel's law is then violated. A composite view of the vector relationship for $\mathbf{h}$ and $-\mathbf{h}$ can be obtained, as in Fig. 2.4.3.3, by reflecting the vectors corresponding to $-\mathbf{h}$ about the real axis of the vector diagram. $\mathbf{F}_P$ and $\mathbf{F}_Q$ corresponding to the two reflections superpose exactly, but $\mathbf{F}_Q''$ do not. $\mathbf{F}_N(+)$ and $\mathbf{F}_N(-)$ then have different magnitudes and phases.

It is easily seen that Friedel's law is obeyed in centric data even when anomalous scatterers are present. $\mathbf{F}_P$ and $\mathbf{F}_Q$ are then parallel to the real axis and $\mathbf{F}_Q''$ perpendicular to it. The vector sum of the three components is the same for $\mathbf{h}$ and $-\mathbf{h}$. It may, however, be noted that the phase angle of the structure factor is then no longer 0 or 180°. Even when the structure is noncentrosymmetric, the effect of anomalous scattering in terms of intensity differences between Friedel equivalents varies from reflection to reflection. The difference between $\mathbf{F}_N(+)$ and $\mathbf{F}_N(-)$ is zero when $\alpha_P = \alpha_Q$ or $\alpha_Q + 180°$. The difference tends to the maximum possible value $(2F_Q'')$ when $\alpha_P = \alpha_Q \pm 90°$.

Intensity differences between Friedel equivalents depend also on the ratio (in terms of number and scattering power) between anomalous and normal scatterers. Differences obviously do not occur when all the atoms are normal scatterers. On the other hand, a structure containing only anomalous scatterers of the same type also

Fig. 2.4.3.2. Vector diagram illustrating the violation of Friedel's law when $\mathbf{F}_Q'' \neq 0$.

does not give rise to intensity differences. Expressions for intensity differences between Friedel equivalents have been derived by Zachariasen (1965) for the most general case of a structure containing normal as well as different types of anomalous scatterers. Statistical distributions of such differences under various conditions have also been derived (Parthasarathy & Srinivasan, 1964; Parthasarathy, 1967). It turns out that, with a single type of anomalous scatterer in the structure, the ratio

$$\frac{|F_N^2(+) - F_N^2(-)|}{F_N^2(+) + F_N^2(-)}$$

has a maximum mean value when the scattering powers of the anomalous scatterers and the normal scatterers are nearly the same (Srinivasan, 1972). Also, for a given ratio between the scattering powers, the smaller the number of anomalous scatterers, the higher is the mean ratio.

Fig. 2.4.3.3. A composite view of the vector relationship between $\mathbf{F}_N(+)$ and $\mathbf{F}_N(-)$.

2.4.3.3. Friedel and Bijvoet pairs

The discussion so far has been concerned essentially with crystals belonging to space groups $P1$ and $P\bar{1}$. In the centrosymmetric space group, the crystal and the diffraction pattern have the same symmetry, namely, an inversion centre. In $P1$, however, the crystals are noncentrosymmetric while the diffraction pattern has an inversion centre, in the absence of anomalous scattering. When anomalous scatterers are present in the structure ($\mathbf{F}_Q'' \neq 0$), Friedel's law breaks down and the diffraction pattern no longer has an inversion centre. Thus the diffraction pattern displays the same symmetry as that of the crystal in the presence of anomalous scattering. The same is true with higher-symmetry space groups also. For example, consider a crystal with space group $P222$, containing anomalous scatterers. The magnitudes of $\mathbf{F}_P$ are the same for all equivalent reflections; so are those of $\mathbf{F}_Q$ and $\mathbf{F}_Q''$. Their phase angles, however, differ from one equivalent to another, as can be seen from Table 2.4.3.1. When $\mathbf{F}_Q'' = 0$, the magnitudes of the vector sum of $\mathbf{F}_P$ and $\mathbf{F}_Q$ are the same for all the equivalent reflections. The intensity pattern thus has point-group symmetry $2/m\ 2/m\ 2/m$. When $\mathbf{F}_Q'' \neq 0$, the equivalent reflections can be grouped into two sets in terms of their intensities: hkl, $h\bar{k}\bar{l}$, $\bar{h}k\bar{l}$ and $\bar{h}\bar{k}l$; and $\bar{h}\bar{k}\bar{l}$, $\bar{h}kl$, $h\bar{k}l$ and $hk\bar{l}$. The equivalents belonging to the first group have the same intensity; so have the equivalents in the second group. But the two intensities are different. Thus the symmetry of the pattern is 222, the same as that of the crystal.

In general, under conditions of anomalous scattering, equivalent reflections generated by the symmetry elements in the crystal have intensities different from those of equivalent reflections generated by the introduction of an additional inversion centre in normal scattering. There have been suggestions that a reflection from the first group and another from the second group should be referred to as a 'Bijvoet pair' instead of a 'Friedel pair', when the two reflections are not inversely related. Most often, however, the terms are used synonymously. The same practice will be followed in this article.

2.4.3.4. Determination of absolute configuration

The determination of the absolute configuration of chiral molecules has been among the most important applications of anomalous scattering. Indeed, anomalous scattering is the only effective method for this purpose and the method, first used in the early 1950s (Peerdeman *et al.*, 1951), has been extensively employed in structural crystallography (Ramaseshan, 1963; Vos, 1975).

Many molecules, particularly biologically important ones, are chiral in that the molecular structure is not superimposable on its mirror image. Chirality (handedness) arises primarily on account of the presence of asymmetric carbon atoms in the molecule. A tetravalent carbon is asymmetric when the four atoms (or groups) bonded to it are all different from one another. The substituents can then have two distinct arrangements which are mirror images of (or related by inversion to) each other. These optical isomers or enantiomers have the same chemical and physical properties except
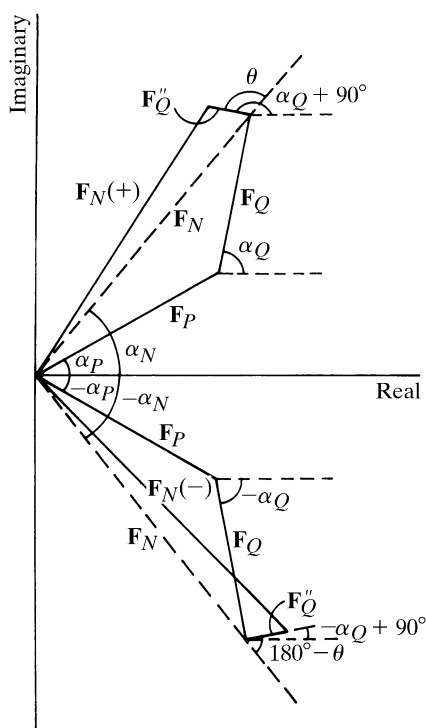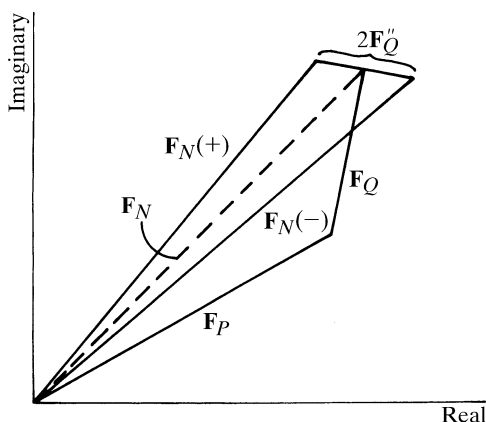
Table 2.4.3.1. *Phase angles of different components of the structure factor in space group $P222$*

	Phase angle (°) of		
Reflection	$\mathbf{F}_P$	$\mathbf{F}_Q$	$\mathbf{F}_Q''$
hkl, $h\bar{k}\bar{l}$, $\bar{h}k\bar{l}$, $\bar{h}\bar{k}l$	α_P	α_Q	$90 + \alpha_Q$
$\bar{h}\bar{k}\bar{l}$, $\bar{h}kl$, $h\bar{k}l$, $hk\bar{l}$	$-\alpha_P$	$-\alpha_Q$	$90 - \alpha_Q$

that they rotate the plane of polarization in opposite directions when polarized light passes through them. It is not, however, possible to calculate the sign of optical rotation, given the exact spatial arrangement or the 'absolute configuration' of the molecule. Therefore, one cannot distinguish between the possible enantiomorphic configurations of a given asymmetric molecule from measurements of optical rotation. This is also true of molecules with chiralities generated by overall asymmetric geometry instead of the presence of asymmetric carbon atoms in them.

Normal X-ray scattering does not distinguish between enantiomers. Two structures A (x_j, y_j, z_j) and B $(-x_j, -y_j, -z_j)$ $(j = 1, \ldots, N)$ obviously produce the same diffraction pattern on account of Friedel's law. The situation is, however, different when anomalous scatterers are present in the structure. The intensity difference between reflections $\mathbf{h}$ and $-\mathbf{h}$, or that between members of any Bijvoet pair, has the same magnitude, but opposite sign for structures A and B. If the atomic coordinates are known, the intensities of Bijvoet pairs can be readily calculated. The absolute configuration can then be determined, *i.e.* one can distinguish between A and B by comparing the calculated intensities with the observed ones for a few Bijvoet pairs with pronounced anomalous effects.

2.4.3.5. *Determination of phase angles*

An important application of anomalous scattering is in the determination of phase angles using Bijvoet differences (Ramachandran & Raman, 1956; Peerdeman & Bijvoet, 1956). From Figs. 2.4.3.2 and 2.4.3.3, we have

$$F_N^2(+) = F_N^2 + F_Q''^2 + 2F_N F_Q'' \cos \theta \qquad (2.4.3.3)$$

and

$$F_N^2(-) = F_N^2 + F_Q''^2 - 2F_N F_Q'' \cos \theta. \qquad (2.4.3.4)$$

Then

$$\cos \theta = \frac{F_N^2(+) - F_N^2(-)}{4F_N F_Q''}. \qquad (2.4.3.5)$$

In the above equations F_N may be approximated to $[F_N(+) + F_N(-)]/2$. Then θ can be evaluated from (2.4.3.5) except for the ambiguity in its sign. Therefore, from Fig. 2.4.3.2, we have

$$\alpha_N = \alpha_Q + 90° \pm \theta. \qquad (2.4.3.6)$$

The phase angle thus has two possible values symmetrically distributed about $\mathbf{F}_Q''$. Anomalous scatterers are usually heavy atoms and their positions can most often be determined by Patterson methods. α_Q can then be calculated and the two possible values of α_N for each reflection evaluated using (2.4.3.6).

In practice, the twofold ambiguity in phase angles can often be resolved in a relatively straightforward manner. As indicated earlier, anomalous scatterers usually have relatively high atomic numbers. The 'heavy-atom' phases calculated from their positions therefore contain useful information. For any given reflection, that phase angle which is closer to the heavy-atom phase, from the two phases calculated using (2.4.3.6), may be taken as the correct phase angle. This method has been successfully used in several structure determinations including that of a derivative of vitamin B_{12} (Dale *et al.*, 1963). The same method was also employed in a probabilistic fashion in the structure solution of a small protein (Hendrickson & Teeter, 1981). A method for obtaining a unique, but approximate, solution for phase angles from (2.4.3.6) has also been suggested (Srinivasan & Chacko, 1970). An accurate unique solution for phase angles can be obtained if one collects two sets of intensity data using two different wavelengths which have different dispersion-correction terms for the anomalous scatterers in the structure. Two

equations of the type (2.4.3.6) are then available for each reflection and the solution common to both is obviously the correct phase angle. Different types of Patterson and Fourier syntheses can also be employed for structure solution using intensity differences between Bijvoet equivalents (Srinivasan, 1972; Okaya & Pepinsky, 1956; Pepinsky *et al.*, 1957).

An interesting situation occurs when the replaceable atoms in a pair of isomorphous structures are anomalous scatterers. The phase angles can then be uniquely determined by combining isomorphous replacement and anomalous-scattering methods. Such situations normally occur in protein crystallography and are discussed in Section 2.4.4.5.

2.4.3.6. *Anomalous scattering without phase change*

The phase determination, and hence the structure solution, outlined above relies on the imaginary component of the dispersion correction. Variation in the real component can also be used in structure analysis. In early applications of anomalous scattering, the real component of the dispersion correction was made use of to distinguish between atoms of nearly the same atomic numbers (Mark & Szillard, 1925; Bradley & Rodgers, 1934). For example, copper and manganese, with atomic numbers 29 and 25, respectively, are not easily distinguishable under normal X-ray scattering. However, the real components of the dispersion correction for the two elements are -1.129 and -3.367, respectively, when Fe $K\alpha$ radiation is used (*IT* IV, 1974). Therefore, the difference between the scattering factors of the two elements is accentuated when this radiation is used. The difference is more pronounced at high angles as the normal scattering factor falls off comparatively rapidly with increasing scattering angle whereas the dispersion-correction term does not.

The structure determination of $KMnO_4$ provides a typical example for the use of anomalous scattering without phase change in the determination of a centrosymmetric structure (Ramaseshan *et al.*, 1957; Ramaseshan & Venkatesan, 1957). f' and f'' for manganese for Cu $K\alpha$ radiation are -0.568 and 2.808, respectively. The corresponding values for Fe $K\alpha$ radiation are -3.367 and 0.481, respectively (*IT* IV, 1974). The data sets collected using the two radiations can now be treated as those arising from two perfectly isomorphous crystals. The intensity differences between a reflection in one set and the corresponding reflection in the other are obviously caused by the differences in the dispersion-correction terms. They can, however, be considered formally as intensity differences involving data from two perfectly isomorphous crystals. They can be used, as indeed they were, to determine the position of the manganese ion through an appropriate Patterson synthesis (see Section 2.4.4.2) and then to evaluate the signs of structure factors using (2.4.2.6) when the structure is centrosymmetric. When the structure is noncentrosymmetric, a twofold ambiguity exists in the phase angles in a manner analogous to that in the isomorphous replacement method. This ambiguity can be removed if the structure contains two different subsets of atoms $Q1$ and $Q2$ which, respectively, scatter radiations λ_{Q1} and λ_{Q2} anomalously. Data sets can then be collected with λ, which is scattered normally by all atoms, λ_{Q1} and λ_{Q2}. The three sets can be formally treated as those from three perfectly isomorphous structures and the phase determination effected using (2.4.2.7) (Ramaseshan, 1963).

2.4.3.7. *Treatment of anomalous scattering in structure refinement*

The effect of anomalous scattering needs to be taken into account in the refinement of structures containing anomalous scatterers, if accurate atomic parameters are required. The effect of the real part of the dispersion correction is largely confined to the thermal parameters of anomalous scatterers. This effect can be eliminated

by simply adding f' to the normal scattering factor of the anomalous scatterers.

The effects of the imaginary component of the dispersion correction are, however, more complex. These effects could lead to serious errors in positional parameters when the space group is polar, if data in the entire diffraction sphere are not used (Ueki *et al.*, 1966; Cruickshank & McDonald, 1967). For example, accessible data in a hemisphere are normally used for X-ray analysis when the space group is $P1$. If the hemisphere has say h positive, the x coordinates of all the atoms would be in error when the structure contains anomalous scatterers. The situation in other polar space groups has been discussed by Cruickshank & McDonald (1967). In general, in the presence of anomalous scattering, it is desirable to collect data for the complete sphere, if accurate structural parameters are required (Srinivasan, 1972).

Methods have been derived to correct for dispersion effects in observed data from centrosymmetric and noncentrosymmetric crystals (Patterson, 1963). The methods are empirical and depend upon the refined parameters at the stage at which corrections are applied. This is obviously an unsatisfactory situation and it has been suggested that the measured structure factors of Bijvoet equivalents should instead be treated as independent observations in structure refinement (Ibers & Hamilton, 1964). The effect of dispersion corrections needs to be taken into account to arrive at the correct scale and temperature factors also (Wilson, 1975; Gilli & Cruickshank, 1973).

2.4.4. Isomorphous replacement and anomalous scattering in protein crystallography

2.4.4.1. *Protein heavy-atom derivatives*

Perhaps the most spectacular applications of isomorphous replacement and anomalous-scattering methods have been in the structure solution of large biological macromolecules, primarily proteins. Since its first successful application on myoglobin and haemoglobin, the isomorphous replacement method, which is often used in conjunction with the anomalous-scattering method, has been employed in the solution of scores of proteins. The application of this method involves the preparation of protein heavy-atom derivatives, *i.e.* the attachment of heavy atoms like mercury, uranium and lead, or chemical groups containing them, to protein crystals in a coherent manner without changing the conformation of the molecules and their crystal packing. This is only rarely possible in ordinary crystals as the molecules in them are closely packed. Protein crystals, however, contain large solvent regions and isomorphous derivatives can be prepared by replacing the disordered solvent molecules by heavy-atom-containing groups without disturbing the original arrangement of protein molecules.

2.4.4.2. *Determination of heavy-atom parameters*

For any given reflection, the structure factor of the native protein crystal $(\mathbf{F}_N)$, that of a heavy-atom derivative $(\mathbf{F}_{NH})$, and the contribution of the heavy atoms in that derivative $(\mathbf{F}_H)$ are related by the equation

$$\mathbf{F}_{NH} = \mathbf{F}_N + \mathbf{F}_H. \qquad (2.4.4.1)$$

The value of $\mathbf{F}_H$ depends not only on the positional and thermal parameters of the heavy atoms, but also on their occupancy factors, because, at a given position, the heavy atom may not often be present in all the unit cells. For example, if the heavy atom is present at a given position in only half the unit cells in the crystal, then the occupancy factor of the site is said to be 0.5.

For the successful determination of the heavy-atom parameters, as also for the subsequent phase determination, the data sets from the native and the derivative crystals should have the same relative scale. The different data sets should also have the same overall temperature factor. Different scaling procedures have been suggested (Blundell & Johnson, 1976) and, among them, the following procedure, based on Wilson's (1942) statistics, appears to be the most feasible in the early stages of structure analysis.

Assuming that the data from the native and the derivative crystals obey Wilson's statistics, we have, for any range of $\sin^2 \theta / \lambda^2$,

$$\ln \left\{ \frac{\sum f_{Nj}^2}{\langle F_N^2 \rangle} \right\} = \ln K_N + 2B_N \frac{\sin^2 \theta}{\lambda^2} \qquad (2.4.4.2)$$

and

$$\ln \left\{ \frac{\sum f_{Nj}^2 + \sum f_{Hj}^2}{\langle F_{NH}^2 \rangle} \right\} = \ln K_{NH} + 2B_{NH} \frac{\sin^2 \theta}{\lambda^2}, \qquad (2.4.4.3)$$

where f_{Nj} and f_{Hj} refer to the atomic scattering factors of protein atoms and heavy atoms, respectively. K_N and K_{NH} are the scale factors to be applied to the intensities from the native and the derivative crystals, respectively, and B_N and B_{NH} the temperature factors of the respective structure factors. Normally one would be able to derive the absolute scale factor and the temperature factor for both the data sets from (2.4.4.2) and (2.4.4.3) using the well known Wilson plot. The data from protein crystals, however, do not follow Wilson's statistics as protein molecules contain highly non-random features. Therefore, in practice, it is difficult to fit a straight line through the points in a Wilson plot, thus rendering the parameters derived from it unreliable. (2.4.4.2) and (2.4.4.3) can, however, be used in a different way. From the two equations we obtain

$$\ln \left\{ \frac{\sum f_{Nj}^2 + \sum f_{Hj}^2}{\sum f_{Nj}^2} \cdot \frac{\langle F_N^2 \rangle}{\langle F_{NH}^2 \rangle} \right\}$$
$$= \ln \left(\frac{K_{NH}}{K_N} \right) + 2(B_{NH} - B_N) \frac{\sin^2 \theta}{\lambda^2}. \qquad (2.4.4.4)$$

The effects of structural non-randomness in the crystals obviously cancel out in (2.4.4.4). When the left-hand side of (2.4.4.4) is plotted against $(\sin^2 \theta)/\lambda^2$, it is called a comparison or difference Wilson plot. Such plots yield the ratio between the scales of the derivative and the native data, and the additional temperature factor of the derivative data. Initially, the number and the occupancy factors of heavy-atom sites are unknown, and are roughly estimated from intensity differences to evaluate $\sum f_{Hj}^2$. These estimates usually undergo considerable revision in the course of the determination and the refinement of heavy-atom parameters.

At first, heavy-atom positions are most often determined by Patterson syntheses of one type or another. Such syntheses are discussed in some detail elsewhere in Chapter 2.3. They are therefore discussed here only briefly.

Equation (2.4.2.6) holds when the data are centric. F_H is usually small compared to F_N and F_{NH}, and the minus sign is then relevant on the left-hand side of (2.4.2.6). Thus the difference between the magnitudes of $\mathbf{F}_{NH}$ and $\mathbf{F}_N$, which can be obtained experimentally, normally gives a correct estimate of the magnitude of $\mathbf{F}_H$ for most reflections. Then a Patterson synthesis with $(F_{NH} - F_N)^2$ as coefficients corresponds to the distribution of vectors between heavy atoms, when the data are centric. But proteins are made up of L-amino acids and hence cannot crystallize in centrosymmetric space groups. However, many proteins crystallize in space groups with centrosymmetric projections. The centric data corresponding to these projections can then be used for determining heavy-atom positions through a Patterson synthesis of the type outlined above.

references