

2. RECIPROCAL SPACE IN CRYSTAL-STRUCTURE DETERMINATION

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 $B(-x_j, -y_j, -z_j)$ ($j = 1, \dots, N$) 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

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

and

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

Then

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

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

$$\alpha_N=\alpha_Q+90^\circ\pm\theta. \quad (2.4.3.6)$$

The phase angle thus has two possible values symmetrically distributed about 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 B₁₂ (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 KMnO₄ 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 Q_1 and Q_2 which, respectively, scatter radiations λ_{Q_1} and λ_{Q_2} anomalously. Data sets can then be collected with λ , which is scattered normally by all atoms, λ_{Q_1} and λ_{Q_2} . 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