

# The Use of Anomalous Scattering in Crystal Structure Analysis

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I. Determination of the Absolute Configuration of Crystals using Anomalous Scattering Techniques . . . . .	67
A. The Absolute Configuration of a Molecule and its Significance . . . . .	67
B. Friedel's Law and its Failure . . . . .	68
C. Unique Indexing of X-Ray Reflections . . . . .	74
D. The Bijvoet Pairs . . . . .	77
E. Some Problems in the Photography of Bijvoet Pairs . . . . .	79
F. An Example of the Determination of Absolute Configuration . . . . .	80
II. Determination of the Phases of Reflections by Anomalous Scattering . . . . .	83
A. The Use of Anomalous Scattering without Phase Change . . . . .	83
B. The Use of Anomalous Scattering with Phase Change . . . . .	91
References . . . . .	94

## I. Determination of the Absolute Configuration of Crystals using Anomalous Scattering Techniques

### A. THE ABSOLUTE CONFIGURATION OF A MOLECULE AND ITS SIGNIFICANCE

It was in 1874 that van't Hoff and le Bel put forward the theory of the tetrahedral carbon atom. If four different groups of atoms W, X, Y and Z are attached to the carbon atom C, the tetrahedral molecule C (W, X, Y, Z) can exist in two forms shown in Fig. 1 and the carbon atom is said to be an asymmetric centre. These two non-superposable enantiomorphous forms are called optical isomers. Both are optically active, one being dextro-rotatory while the other is laevo-rotatory. From the point of view of optical theory it is of interest to know which of the two spatial arrangements is dextro- and which is laevo-rotatory. It is indeed a sad commentary on our present knowledge of optical phenomena that, given the exact spatial arrangement or the absolute configuration of a molecule, it is not possible to compute with certainty the sign of its optical rotation (see Beurskens-Kerssen *et al.*, 1963).

The determination of the absolute configuration is of significance because of the vital role optical isomerism plays in life's processes. To quote the simplest example, while dextro-rotatory glucose is almost

instantaneously absorbed by the human system, the laevo form is rejected by it. The synthetic-drug chemist must know the absolute configuration of the isomer of the compound which is physiologically active so that he may attempt at a specific synthesis of this particular form. The problem of how certain enzymes achieve specific synthesis of certain optical isomers, and also the more general questions of the biogenesis of optically active compounds in plants and animals, are basic problems to the organic chemist and the biochemist.

The classical work of Werner has extended the concept of optical isomerism to inorganic complexes and there can be no doubt that the determination of absolute configuration of optically active Werner

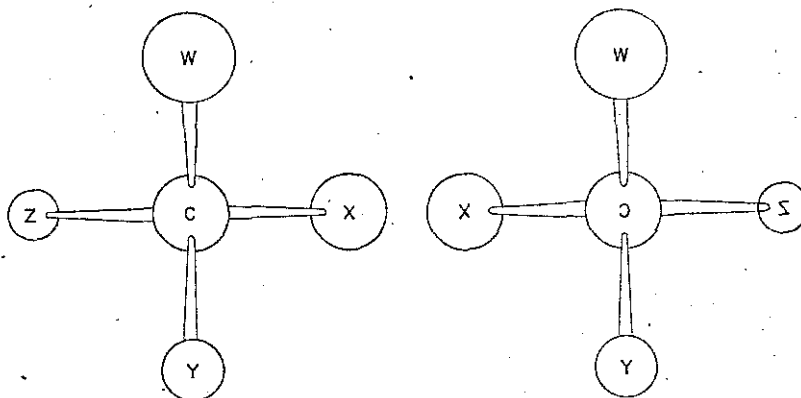


Fig. 1. Optical isomerism due to the tetrahedral carbon atom.

complexes would extend the frontiers of inorganic synthesis. The determination of the absolute configuration of substances which are optically active only in the crystalline state is a necessary step before one really understands either the phenomenon of optical activity or the crystalline state.

After this brief statement of the significance of the absolute configuration of a molecule to physics and chemistry it may be of some gratification to the X-ray crystallographer to know that the only effective method by which it may be established unambiguously is that proposed by Bijvoet (1949), of using anomalous scattering of X-rays.

## B. FRIEDEL'S LAW AND ITS FAILURE

### 1. Normal scattering

If two structures *A* and *B* are enantiomorphous and if

$$\text{structure } A \equiv (x_j, y_j, z_j) \quad (j = 1 \dots N) \quad (1a)$$

then

$$\text{structure } B \equiv (\bar{x}_j, \bar{y}_j, \bar{z}_j) \quad (j = 1 \dots N) \quad (1b)$$

If for simplicity we assume that the two structures belong to space group  $P1$ , their structure factors are given by

$$F_A(hkl) = \sum_{j=1}^N f_j \exp i\alpha_j = |F(hkl)| \exp i\alpha \quad (2a)$$

$$F_B(hkl) = \sum_{j=1}^N f_j \exp -i\alpha_j = |F(hkl)| \exp -i\alpha \quad (2b)$$

For the inverse reflections  $\bar{h}\bar{k}\bar{l}$  the respective structure factors are

$$F_A(\bar{h}\bar{k}\bar{l}) = \sum_{j=1}^N f_j \exp -i\alpha_j = |F(hkl)| \exp -i\alpha \quad (3a)$$

$$F_B(\bar{h}\bar{k}\bar{l}) = \sum_{j=1}^N f_j \exp i\alpha_j = |F(hkl)| \exp i\alpha \quad (3b)$$

Hence for the normal scattering of X-rays

$$|F_A(hkl)| = |F_B(hkl)| = |F_A(\bar{h}\bar{k}\bar{l})| = |F_B(\bar{h}\bar{k}\bar{l})| \quad (4a)$$

$$\alpha_A(hkl) = -\alpha_B(hkl) = -\alpha_A(\bar{h}\bar{k}\bar{l}) = \alpha_B(\bar{h}\bar{k}\bar{l}) \quad (4b)$$

and

$$I_A(hkl) = I_A(\bar{h}\bar{k}\bar{l}) = I_B(hkl) = I_B(\bar{h}\bar{k}\bar{l}) \quad (4c)$$

It must be noted that in a non-centrosymmetric crystal the sequence of atoms which the X-ray beam encounters from the side  $hkl$  is opposite to and therefore different from that met by it when incident from the side  $\bar{h}\bar{k}\bar{l}$ . Even so, according to (4c), from the measurement of the intensities of various reflections from a crystal it would be impossible to determine which of the two enantiomorphs ( $A$  or  $B$ ) is the correct structure. The equality given in (4c) is known as Friedel's law.

## 2. Anomalous dispersion effects

The impasse of not being able to distinguish the optical or electrical antipodes by X-ray methods was broken when Coster *et al.* (1930) discovered that Friedel's law was violated when the structure contained at least two sets of atoms, one of which scatters the incident X-rays anomalously. When the wavelength of the incident X-ray beam is close to the absorption edge of a scattering atom, the atomic scattering factor becomes a complex number expressible as

$$f = f_0 + \Delta f' + i\Delta f'' \quad (5)$$

where  $f_0$  is the normal scattering factor for wavelengths very far from the absorption edge, and  $\Delta f'$  and  $\Delta f''$  are the correction terms which arise due to dispersion effects. The quantity  $\Delta f'$  is usually negative and  $\Delta f''$ , the imaginary part, is always ahead of the real part ( $f_0 + \Delta f'$ )

by  $\pi/2$ . Further,  $\Delta f'' = 0$  for  $\nu_i < \nu_{\text{abs. edge}}$ . Figure 2 gives qualitatively the variation of  $\Delta f'$  and  $\Delta f''$  with the position of the incident frequency with respect to an absorption edge. The two correction factors  $\Delta f'$  and  $\Delta f''$  may be considered to be independent of the scattering angle as the electrons responsible for these effects are confined to a very small volume near the nucleus. (However, see Hunter, 1958, 1959 a, b; Batterman, 1962.)

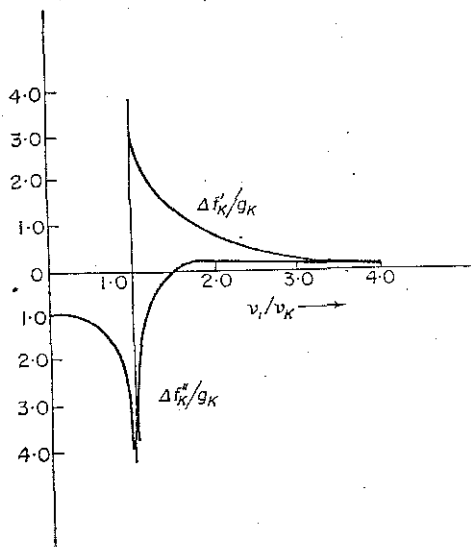


FIG. 2. Anomalous dispersion effects near a  $K$  absorption edge.  $\nu_i$  is the incident frequency and  $\nu_K$  the frequency of the  $K$  edge.  $\Delta f'' \neq 0$  only when  $\nu_i > \nu_K$ .  $g_K$  is the oscillator strength of the  $K$  electrons (Hönl, 1933).

The quantity  $\Delta f''$  is scattered  $\pi/2$  ahead of the phase of the real part ( $f_0 + \Delta f'$ ) and this advance in phase is independent of the direction of incidence of the X-ray beam and of the position of atoms in the structure. In consequence, if the structure contains one set of atoms ( $R$ ) which scatters the incident X-rays normally and another set ( $A$ ) which scatters it anomalously, the amplitudes and phases which arise due to  $f_R$ ,  $f_{A0}$ ,  $\Delta f'_A$  and  $\Delta f''_A$  for the two reflections  $hkl$  and  $\bar{h}\bar{k}\bar{l}$  are respectively

$$\begin{array}{llll} hkl & |F_R|, \alpha_R; & |F_{A0}|, \alpha_A; & |\Delta F'|, \alpha_A; & |F''|, \alpha_A + \pi/2 \\ \bar{h}\bar{k}\bar{l} & |F_R|, -\alpha_R; & |F_{A0}|, -\alpha_A; & |\Delta F'|, -\alpha_A; & |F''|, -\alpha_A + \pi/2 \end{array}$$

This asymmetry in the phase of scattering causes the resultant structure amplitudes to be different in the two cases (see Fig. 3(c)), so that

$$\begin{aligned} |F(hkl)| &\neq |F(\bar{h}\bar{k}\bar{l})| \\ I(hkl) &\neq I(\bar{h}\bar{k}\bar{l}) \end{aligned} \quad (6)$$

and

$$\alpha(hkl) \neq -\alpha(\bar{h}\bar{k}\bar{l})$$

It must be noted that Friedel's law breaks down only if anomalous scattering with phase change exists ( $\Delta f'' \neq 0$ ). It does not break down when there is anomalous scattering without phase change ( $\Delta f' \neq 0$  and  $\Delta f'' = 0$ ). This is illustrated in Fig. 3.

In the case of ZnS using  $\text{AuL}\alpha(1.2738\text{\AA})$  to excite the Zn atom

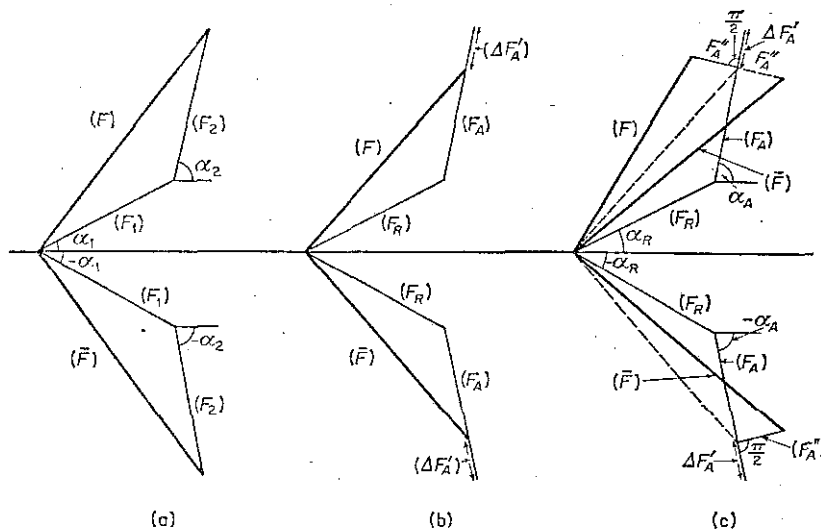


FIG. 3. The structure amplitude of  $hkl(F)$  and  $hkl(\bar{F})$  under normal and anomalous scattering. (a)  $\Delta f' = 0$ ,  $\Delta f'' = 0$ ; (b)  $\Delta f' \neq 0$ ,  $\Delta f'' = 0$ ; (c)  $\Delta f' \neq 0$ ,  $\Delta f'' \neq 0$ . There are two sets of atoms in the structure,  $B$  (normal scatterers) and  $A$  (anomalous scatterers). Friedel's law breaks down only when  $\Delta f'' \neq 0$ .

( $\lambda_{\text{K edge}} = 1.2805\text{\AA}$ ) Coster, Knol and Prins demonstrated the unequal intensities of the 111 and  $\bar{1}\bar{1}\bar{1}$  reflections. Although these workers had, in effect, detected the asymmetry of the sequence of atoms for the two planes (111) and ( $\bar{1}\bar{1}\bar{1}$ ) in ZnS, its deeper implication to molecular structure was not appreciated till Bijvoet (1949) and his co-workers (Peerdeman *et al.*, 1951) used this technique for the determination of the absolute configuration of sodium rubidium tartrate whose structure had already been determined. Using  $\text{ZrK}\alpha$  ( $\lambda = 0.786\text{\AA}$ ), they observed significant differences between  $hkl$  and  $\bar{h}\bar{k}\bar{l}$  reflections. Knowing the atomic co-ordinates, the amplitudes  $|F(hkl)|$  and  $|F(\bar{h}\bar{k}\bar{l})|$  to be expected for the two enantiomorphs were calculated. Experimentally it was determined whether  $I(hkl)$  was greater or less than  $I(\bar{h}\bar{k}\bar{l})$ . A comparison

of the experimentally observed inequality with the computed one permitted the absolute configuration to be determined uniquely.

In view of this pioneering work of Bijvoet, we shall call a Bijvoet pair a pair of reflections whose intensities are equal if  $\Delta f'' = 0$  but become unequal when  $\Delta f'' \neq 0$ .

### 3. The proper choice of the incident radiation and the optimum conditions

Peterson (1955) and Templeton (1955) pointed out that even if the incident wavelength is on the longer side of the  $K$  absorption edge it may have sufficient energy to excite the  $L$  or even the  $M$  electrons.

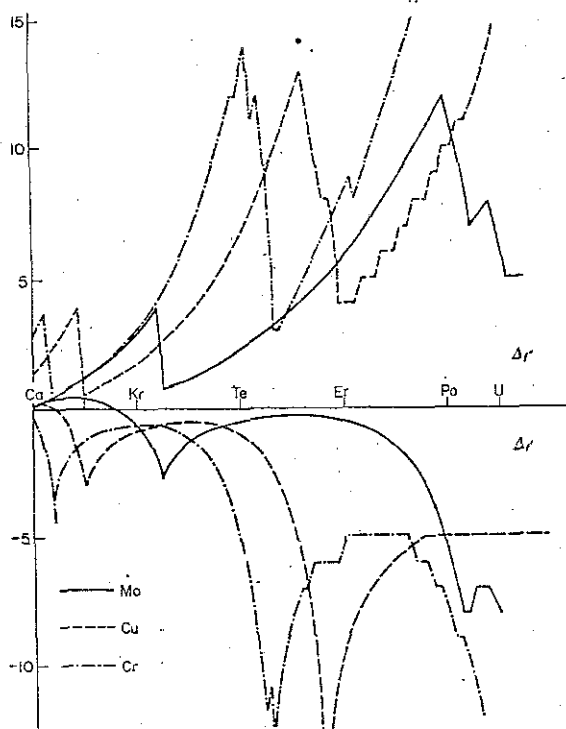


FIG. 4. The variation of  $\Delta f'$  and  $\Delta f''$  with atomic number for  $\text{MoK}\alpha$ ,  $\text{CuK}\alpha$  and  $\text{CrK}\alpha$  (after Dauben and Templeton, 1955).

This results in significant anomalous scattering with phase change even at wavelengths far removed from the  $K$  absorption edge. Using the method of Parratt and Hempstead (1954) the values of  $\Delta f'$  and  $\Delta f''$  for the atoms with  $Z = 20$  to 96 have been computed by Dauben and Templeton (1955) for the three wavelengths  $\text{CrK}\alpha$ ,  $\text{CuK}\alpha$  and  $\text{MoK}\alpha$ . These results are shown in Fig. 4 and it is apparent from it that

commonly available wavelengths may be used for the determination of the absolute configuration provided proper anomalous scatterers are present in the structure.

Since the success of the Bijvoet technique depends on detecting small differences in the intensity between  $I(hkl)$  and  $I(\bar{h}\bar{k}\bar{l})$ ,  $\Delta f''$  for the anomalous scatterer must be as large as possible. Table I gives the range of atoms which may act as anomalous scatterers for the three commonly available radiations. Okaya and Pepinsky (1961) have given a list of useful radiations for various anomalous scatterers.

TABLE I

Range of convenient anomalous scatterers for  $CrK\alpha$ ,  $CuK\alpha$  and  $MoK\alpha$

Target	Radiation	Range of atoms with $\Delta f'' \geq 2$
Cr (24)	$K\alpha_1$ 2.290Å	As (33)–Ba (56)
	$K\alpha_2$ 2.293Å	
Cu (29)	$K\alpha_1$ 1.540Å	Sr (38)–Dy (60)
	$K\alpha_2$ 1.544Å	
Mo (42)	$K\alpha_1$ 0.709Å	Sb (51)–Rn (86)
	$K\alpha_2$ 0.713Å	

Since  $\Delta f''$  is practically independent of the angle of scattering, the anomalous scattering effects are more prominent at comparatively larger angles. In fact these are sometimes so large that the differences in the intensity of the  $hkl$  and  $\bar{h}\bar{k}\bar{l}$  reflections may be easily detected by direct inspection of Weissenberg photographs. When  $\Delta f''$  is small, Geiger counter techniques are necessary.

Even if a proper radiation is chosen, some pairs of reflections will exhibit intensity differences more prominently than others. Figure 5

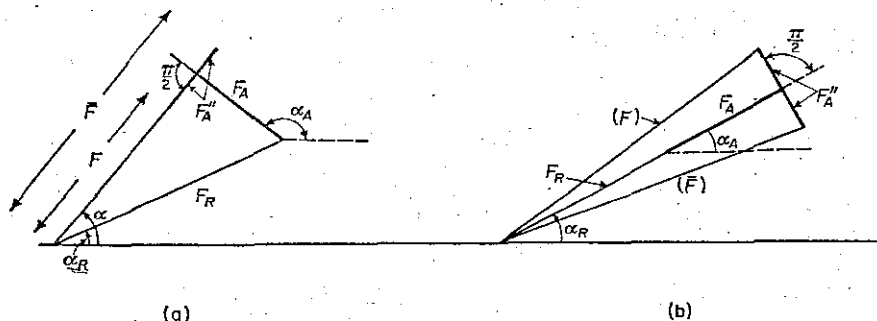


FIG. 5. The difference in the amplitudes of a Bijvoet pair ( $|F| - |\bar{F}|$ ) is (a) maximum and equal to  $2F''_A$  when  $\alpha_R = \alpha_A \pm \pi/2$  and (b) is zero when  $\alpha_R = \alpha_A$  or  $\alpha_A + \pi$ .

shows the conditions under which the intensity difference is a maximum (Fig. 5(a)) or a minimum (Fig. 5(b)). It is also obvious that if a structure contains only one set of atoms it would not be possible to determine the absolute configuration.

### C. UNIQUE INDEXING OF X-RAY REFLECTIONS

The correct indexing of X-ray reflections is extremely important for the determination of the absolute configuration by the Bijvoet method. Since the formulae for the structure amplitudes for any space group are given for a right-handed system of coordinates in the *International Tables for X-ray Crystallography* (1952), great care must be exercised in choosing a proper right-handed system of coordinates while indexing X-ray reflections.

Any error in this procedure would obviously give a wrong absolute configuration.

When a Geiger counter diffractometer is used the unique indexing is quite easy. A right-handed system is assumed and the angular settings at which any plane  $hkl$  and its inverse  $\bar{h}\bar{k}\bar{l}$  reflect are computed. The settings are made so that the reflected X-ray beams enter the counter. The intensities are then directly compared. The indexing of a precession photograph on a right-handed system is also comparatively simple as a precession photograph is an undistorted record of the reciprocal lattice. While indexing higher layers, however, care must be taken to note whether the section made is on the positive or on the negative axis.

The unique indexing in the case of Weissenberg equi-inclination photographs is slightly more complex, but it can be done without any reference whatsoever to the crystal or to its morphological characteristics (Peerdeman and Bijvoet, 1956; Vaidya and Ramaseshan, 1963). Figure 6(a) shows the arrangement in a right-handed camera like the Unicam single crystal goniometer S-35 where the clockwise rotation of the graduated drum head causes the film cassette to move along the direction of advance of a right-handed screw. The original position of the film in the cassette has also to be known and this is achieved by adopting the convention of cutting the top right-hand corner of the film (in relation to the experimenter standing on the side of the camera away from the X-ray tube). The position of the snip on the film when inside the cassette and outside is shown in Fig. 6.

Now imagine a right-handed system of coordinates (Fig. 6(b)) to be superposed on the crystal with the  $c$  axis coinciding with the axis of rotation. By imagining the reflecting sphere of the Ewald construction, it is seen that in a right-handed camera the axial rows will record themselves in the cyclic permutation of the sequence  $h00$ ,  $0k0$ ,  $\bar{h}00$ ,  $0\bar{k}0$  from



right to left on the film (Fig. 6(c)). In the higher layer Weissenberg equi-inclination photographs ( $hkL$ ) also, the same sequence will be obtained. The sense of the rotation of the equi-inclination angle  $\mu$  will decide whether the  $L$  or  $\bar{L}$  layer is being photographed with the chosen coordinate axes. The sense of rotation for positive  $L$  layers is given in Fig. 6(b) for a right-handed camera.

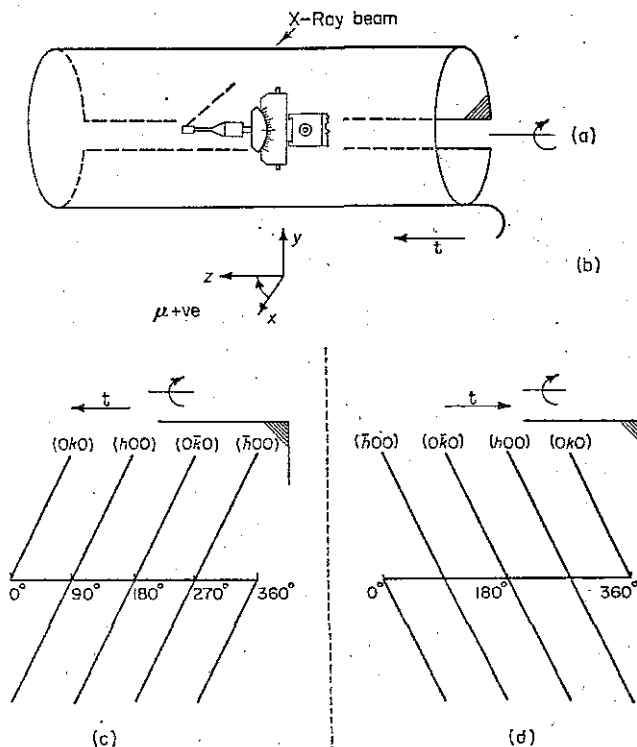


FIG. 6. (a) The direction of the rotation and the translation of a right-handed Weissenberg camera: The direction of the X-ray beam and the position of the cut on the film are also shown. (b) The right-handed system of coordinate axes used for indexing. The direction of  $\mu$  for obtaining positive  $L$  layers is also shown. (c) and (d) The sequence of axial reflections as recorded on a film with a right-handed system of coordinates using (c) a right-handed camera and (d) a left-handed camera. The actual case illustrated is for an orthorhombic crystal including all the axial rows for a rotation of  $360^\circ$ .

Once the above cyclic sequence of axial rows has been chosen on the photograph, one has automatically fixed a right-handed system of coordinates for indexing. This is true whatever be the symmetry of the crystal and it holds for both zero and higher level Weissenberg equi-inclination photographs.

As an illustration, the indexing in the case of a monoclinic crystal

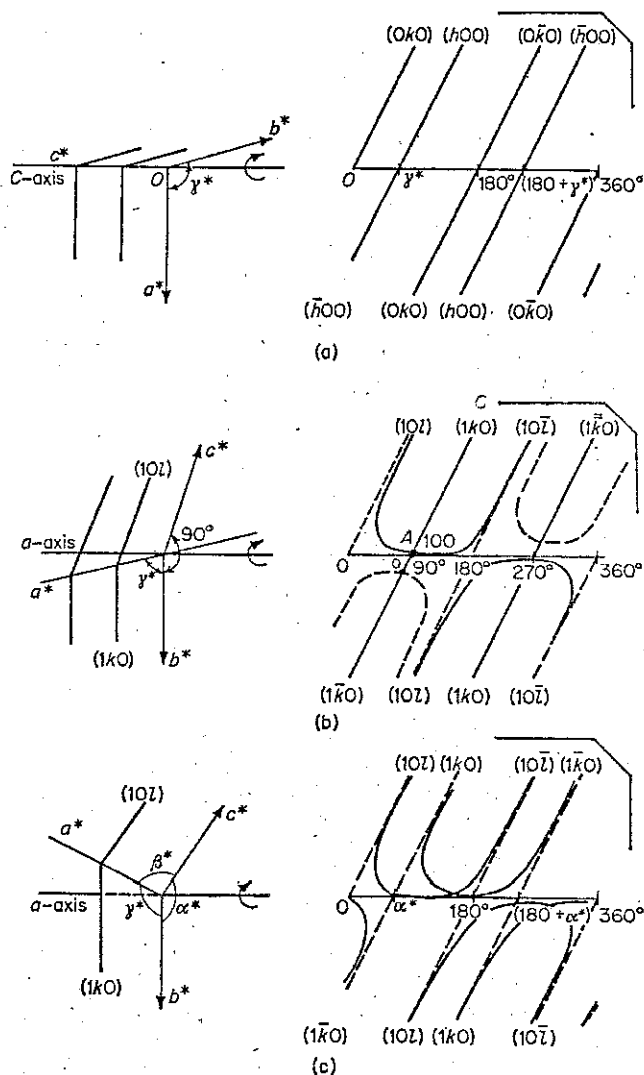


FIG. 7. (a) Zero higher layer equi-inclination Weissenberg photograph of a monoclinic crystal mounted along a unique axis ( $c$ -axis). (b) The higher layer photograph of a monoclinic crystal about the non-unique axis  $a$ . (c) The higher layer photograph of a triclinic crystal mounted about the  $a$ -axis.

(point group 2) may be considered. If the crystal is mounted with its unique axis ( $c$ ) parallel to the axis of rotation the zero layer and higher layer Weissenberg equi-inclination photographs appear as in Fig. 7(a). If the axial rows are indexed as shown, one has chosen a right-handed system of coordinates. Unfortunately for this point group and the unique axis mounting there are no Bijvoet pairs in any equi-inclination

photograph. If the absolute configuration is to be determined using the conventional Weissenberg photography, the crystal has to be mounted about one of its non-unique axes ( $a$  axis, say). With such a mounting the  $0kl$  reflections record themselves in the zero layer, the  $0k0$  and  $00l$  rows appearing as straight lines, displaced  $90^\circ$  from each other. In this photograph, while  $b^*$  and  $c^*$  values may be measured,  $\gamma^*$  cannot be determined. If one decides to ignore the convention of having  $\gamma^*$  always acute, a sequence  $0k0$ ,  $00l$ ,  $0\bar{k}0$ ,  $00\bar{l}$  may be selected from right to left on the photograph which immediately fixes a right-handed system of coordinates, for indexing. On the assumed choice of axes,  $\gamma^*$  may either be acute or obtuse and may be determined by the offset method from higher layer Weissenberg equi-inclination photographs (Buerger, 1942a).

If, on the other hand, one wishes to stick to the convention of choosing  $\gamma^*$  to be acute, then zero layer and higher layer Weissenberg equi-inclination photographs are recorded (the sense of  $\mu$  to correspond to  $+H$ ). In the higher layer equi-inclination photographs the  $Hk0$  reflections record on a straight line while the  $H0l$  reflections fall on a festoon. The distance of the lowest point on the festoon from the central line allows us to compute  $\gamma^*$  (Buerger, 1942a, p. 371, eq. 13). It is quite easy to locate the lowest point of the axial festoon in the case of a monoclinic crystal, as it is the intersection of the  $Hk0$  straight line with the  $H0l$  festoon. (This, however, is not so in the triclinic case.) The angular lag method which gives a more precise value of  $\gamma^*$  may also be used (Buerger, 1942a, p. 380, eq. 18). If the angle determined from one of the minimum points of the  $H0l$  festoon is acute, then  $k$  is positive for the part of the  $Hk0$  line on which this minimum point lies. If the angle is obtuse then  $k$  is negative for this line. With  $+k$  thus chosen, the proper cyclic sequence of axial rows from right to left mentioned earlier fixes a right-handed system of coordinates. For details of indexing a triclinic crystal, reference may be made to the paper by Vaidya and Ramaseshan (1963).

#### D. THE BIJVOET PAIRS

Due to the elements of symmetry possessed by a crystal there may be reflections other than  $hkl$  and  $\bar{h}\bar{k}\bar{l}$  which may also be compared for the determination of the absolute configuration. As mentioned earlier, two reflections which are of equal intensity under normal scattering and which become unequal when anomalous scattering is present may be called a Bijvoet pair. Intensities of Bijvoet pairs must be compared to determine the absolute configuration. The equivalence of reflections from a crystal where normal scattering takes place is governed by the symmetry elements of its Laue group. On the other hand, the equiva-

lent reflections of the same crystal when anomalous scattering is present is obtained by the symmetry elements of its point group. For a crystal belonging to any point group it is easy to work out the Bijvoet pairs using the elements of symmetry of its point group and its Laue group. Conversely, the point group of any crystal may be unambiguously determined, if one uses anomalous scattering techniques (see Ramachandran and Parthasarathy, 1963).

We shall, as an illustration, work out the Bijvoet pairs for the space group  $P2_12_12_1$ . For normal scattering, the X-ray reflections exhibit the Laue group symmetry of  $mmm$ ; for anomalous scattering (with phase

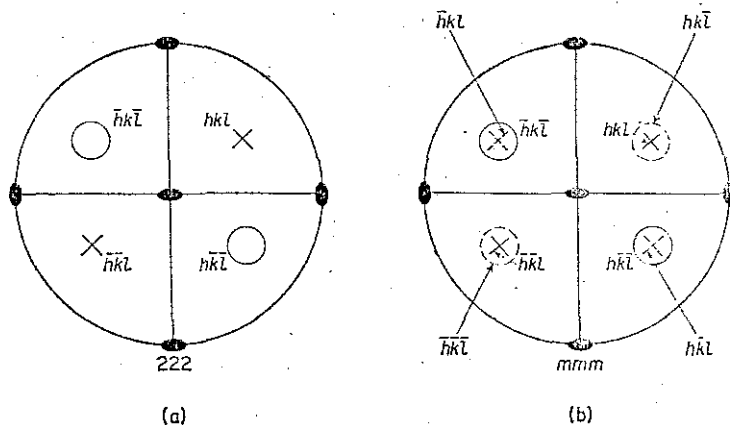


FIG. 8. Stereographic projection for the point group 222 and Laue group  $mmm$ . Equivalent reflections when anomalous scattering is present are shown by continuous lines in (a). The extra-equivalent reflections due to the introduction of a spurious centre of symmetry due to normal scattering are shown by dotted lines in (b). Every reflection represented by continuous lines forms a Bijvoet pair with every one represented by dotted lines.

change) the reflections display the point group symmetry 222. Figure 8(a) shows the equivalent reflections for anomalous scattering (222) and Fig. 8(b) the equivalent reflections under normal scattering ( $mmm$ ). The extra reflections that are made equivalent due to the spurious centre of inversion are marked by dotted lines. It is obvious that any reflection marked by full lines forms a Bijvoet pair with any one marked by dotted lines.

For the point group 222,

$$\begin{aligned} & [|F(hkl)| = |F(\bar{h}k\bar{l})| = |F(h\bar{k}l)| = |F(\bar{h}kl)|] \\ & \neq [|F(\bar{h}kl)| = |F(h\bar{k}l)| = |F(hkl)| = |F(\bar{h}kl)|] \end{aligned}$$

and

$$\alpha(hkl) \neq [-\alpha(\bar{h}k\bar{l}) = -\alpha(\bar{h}kl) = -\alpha(h\bar{k}l) = -\alpha(hkl)]$$

Analytically, the phase relations that may exist amongst the reflections that are equivalent to one another under normal scattering will be of the following types.

Group I	Group II
(a) $\alpha = \beta$	(e) $\alpha = \pi/2 - \beta$
(b) $\alpha = \pi/2 + \beta$	(f) $\alpha = \pi - \beta$
(c) $\alpha = \pi + \beta$	(g) $\alpha = 3\pi/2 - \beta$
(d) $\alpha = 3\pi/2 + \beta$	(h) $\alpha = -\beta$

Even when anomalous scattering is present, reflections in Group I have equal intensity. The reflections in Group II are also of equal intensity but the intensity of those in Group II is different from those in Group I. Hence any reflection in Group I forms a Bijvoet pair with any in Group II. The conditions under which a Bijvoet pair may be accidentally of equal intensity has been worked out (Fig. 5(b)).

#### E. SOME PROBLEMS IN THE PHOTOGRAPHY OF BIJVOET PAIRS

When determining the absolute configuration by comparing the intensities of a Bijvoet pair, it is essential that the specimen be ground into a cylindrical or spherical shape so that the absorption corrections for the two reflections are the same. A second precaution is to avoid, as far as possible, comparing reflections which have been recorded on different sides of the central line in a Weissenberg equi-inclination photograph. Such reflections undergo varying degrees of contraction and elongation and are thus not suitable for comparison if the intensity is measured visually. However, if an integrating Weissenberg Camera is used this difficulty does not arise.

For crystals belonging to the monoclinic, tetragonal and hexagonal classes, there are no Bijvoet pairs in Weissenberg equi-inclination photographs of any layer taken about the unique axis. The reflections to be compared are of the form  $hkl$  and  $h\bar{k}l$  ( $c$  axis unique). These reflections may easily be obtained on one film if the crystal is mounted about a non-unique axis. However, crystals belonging to these classes have a tendency to form needles along the unique axes. Often it is found that it is exceedingly difficult to cut a crystal along a required non-unique axis and grind it into a cylindrical shape. This problem becomes very much more pronounced when one is studying a crystal at low temperatures of a substance which is normally a liquid at room temperature. In low temperature Weissenberg cameras using the Fankuchen-Lipscomb type of cooling the crystal almost invariably grows with a particular crystallographic axis coinciding with the axis of the cylindrical capillary tube. It is not possible to mount the crystal along any other axis.

To obviate these difficulties, simple double layer screens have been devised so that by normal beam Weissenberg photography the reflections of the  $L$  and  $\bar{L}$  layers may be simultaneously recorded on the same film (Beurskens Kerksen *et al.*, 1963; Vaidya and Ramaseshan, 1963). One such is shown in Fig. 9. This double layer method of recording Bijvoet pairs has also the following advantages in the case of crystals with 2-, 4- or 6-fold axes. Both the reflections of the Bijvoet pair  $hkl$  and  $\bar{h}\bar{k}\bar{l}$  will be elongated or contracted by the same extent. The reflections also occur simultaneously so that any fluctuations in the intensity of the incident beam will not matter. In other point groups, or in the usual case of equi-inclination photography where both reflections of a Bijvoet pair are not flashed simultaneously, a stabilized X-ray unit would be necessary.

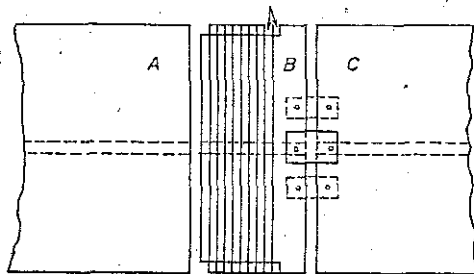


FIG. 9. The double layer screen, in which  $A$  and  $C$  are conventional screen tubes and  $B$  is attached to  $C$  by metal strips. Stepped rings (if necessary magnetized) fit on to  $B$  to give a central screen tube of variable length. (Vaidya and Ramaseshan, 1963.)

#### F. AN EXAMPLE OF THE DETERMINATION OF ABSOLUTE CONFIGURATION

If a structure has been solved using normal procedures then the main steps that are to be followed for the absolute configuration of the molecule to be established are as follows. (a) From the solved structure one of the two possible enantiomorphs (equation (1)) is chosen at random. (b) Using a right-handed system of coordinates, the reflections are uniquely indexed so that there is no ambiguity regarding the  $hkl$  and  $\bar{h}\bar{k}\bar{l}$  reflections. (c) The Bijvoet pairs for the space group are compared and in each available pair it is determined from experiment whether  $I_o(hkl)$  is greater or less than  $I_o(\bar{h}\bar{k}\bar{l})$  (or its equivalent reflection). (d) With the atomic parameters of the chosen enantiomorph, computations are made (either vectorially or trigonometrically) to find out whether  $I_c(hkl)$  is greater or less than  $I_c(\bar{h}\bar{k}\bar{l})$ . (e) The observed inequalities are compared with the calculated ones and, if they agree, the assumed enantiomorph is the correct structure. If not, the inverse of the assumed structure is the correct absolute configuration.

We shall illustrate this by an example from the determination of the absolute configuration of echitamine iodide which crystallizes in the space group  $P2_12_12_1$  (Manohar and Ramaseshan, 1961a). Its structure was solved by the heavy atom technique (Manohar and Ramaseshan, 1961b, 1962) and the atomic parameters were refined to a fair degree of accuracy. Three-dimensional intensity data were collected from the Weissenberg equi-inclination photographs with  $c$  as the rotation axis. There are no Bijvoet pairs in the zero layer photograph for this space group. In the higher layer photograph, the Bijvoet pairs are symmetrically distributed about the  $h0L$  and  $0kL$  axial rows. Actual examination of the photographs showed many pairs of reflections exhibiting significant differences in intensity. For example, it was observed that

$$I(324) < I(3\bar{2}4)$$

For the space group  $P2_12_12_1$  for the group of reflections with

$$(h+k) = 2n+1, (k+l) = 2n$$

the expression for the structure factor is

$$F = A' + iB'$$

where

$$A' = \sum f_j(-4 \sin 2\pi hx_j \cos 2\pi ky_j \sin 2\pi lz_j)$$

and

$$B' = \sum f_j(4 \cos 2\pi hx_j \sin 2\pi ky_j \cos 2\pi lz_j)$$

In this structure the iodine atom scatters  $\text{CuK}\alpha$  radiation anomalously so that  $f_I$  must be replaced by

$$f_0 + \Delta f' + i\Delta f''$$

Thus, for one iodine atom of the asymmetric unit, the structure amplitude is given by

$$\begin{aligned} F &= (f_0 + \Delta f')(-\sin 2\pi hx \cos 2\pi ky \sin 2\pi lz) + \\ &\quad + i(f_0 + \Delta f')(\cos 2\pi hx \sin 2\pi ky \cos 2\pi lz) - \\ &\quad - \Delta f''(\cos 2\pi hx \sin 2\pi ky \cos 2\pi lz) + \\ &\quad + i\Delta f''(-\sin 2\pi hx \cos 2\pi ky \sin 2\pi lz) \\ &= A'_I + iB'_I - A''_I + iB''_I \end{aligned}$$

Knowing the atomic positions of iodine and the scattering factors (for the reflection 324,  $\Delta f' = -1.1$ ,  $\Delta f'' = 7.2$ ) the four terms may be calculated. Similarly the contributions  $A_R$  and  $B_R$  due to the rest of the atoms which scatter normally, are computed using the same formulae (with  $\Delta f' = 0$  and  $\Delta f'' = 0$ ). The resultant  $F(324)$  is obtained graphically. Similarly the components  $\bar{A}'_I$ ,  $\bar{B}'_I$ ,  $\bar{A}''_I$ ,  $\bar{B}''_I$ ,  $\bar{A}_R$  and  $\bar{B}_R$  of the other reflection of the Bijvoet pair (namely  $3\bar{2}4$ ) are calculated.

TABLE II

The components of the structure amplitude for the reflection 324 and  $3\bar{2}4$  of echitamine iodide

$hkl$	$A_R$	$B_R$	$A'_I$	$B'_I$	$A''_I$	$B''_I$	$F_{\text{cal}}$	$I_{\text{cal}}$
324	+9.3	-4.2	-7.7	-8.8	1.5	-1.7	15.0	225
$3\bar{2}4$	+9.3	+4.2	-7.7	+8.8	-1.5	+1.7	11.4	130

Inequality

Calculated	Observed
$I_c(324) > I_c(3\bar{2}4)$	$I_o(324) < I_o(3\bar{2}4)$

These values for the reflections 324 and  $3\bar{2}4$  are given in Table II. The quantities  $F$  and  $\bar{F}$  are determined graphically as in Fig. 10. One finds that  $I_c(324) > I_c(3\bar{2}4)$ , i.e. the computed result is opposite to that determined experimentally. It follows, therefore, that the correct

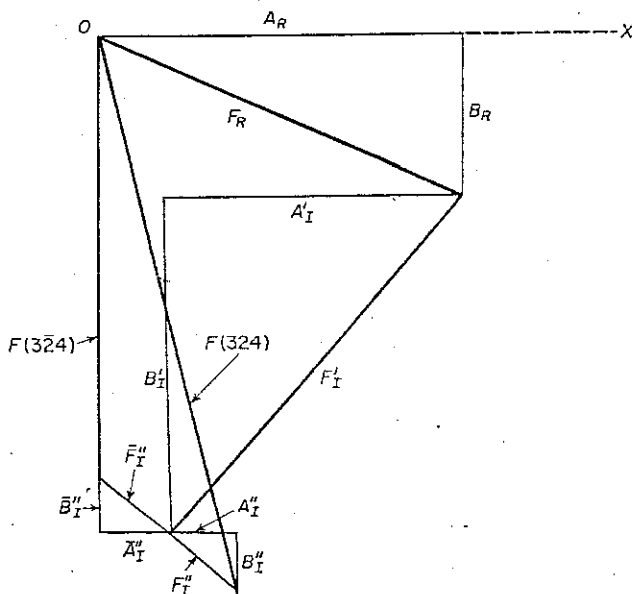


FIG. 10. The vector diagram comparing the structure amplitudes of the Bijvoet pair 324 and  $3\bar{2}4$  in the case of echitamine iodide. ( $C_{22}H_{25}O_4N_2I$ ). (Manohar and Ramaseshan, 1963.)



configuration of the echitamine ion does not correspond to that given by the coordinates used in the calculation of the structure amplitude but its mirror image. Although one pair of reflections is really sufficient to establish the absolute configuration, it is better to compare a larger number of pairs so as to avoid mistakes due to any accidental errors in the recording of the intensities.

## II. Determination of the Phases of Reflections by Anomalous Scattering

### A. THE USE OF ANOMALOUS SCATTERING WITHOUT PHASE CHANGE

#### 1. Distinguishing atoms of nearly equal atomic numbers

For anomalous scattering without phase change,  $\Delta f'' = 0$ , but  $\Delta f'$  may sometimes be as large as 12 or 13 electrons (see Fig. 4). This effect is almost independent of scattering direction. Since normal scattering falls away rapidly with increasing angles of scattering, the relative change due to anomalous scattering increases considerably for large scattering angles. With normal scattering, atoms of nearly equal atomic numbers are difficult to distinguish. If, however, the effective scattering power of one of them is reduced by anomalous scattering, it can easily be identified, the more easily the larger the value of  $\Delta f'$ . This makes anomalous scattering an effective tool in crystal structure analysis to distinguish nearly equal atoms.

Mark and Szillard (1925) first demonstrated this effect strikingly in RbBr, which has a KCl type of structure. The 111 reflection whose structure amplitude is  $f_{\text{Rb}} - f_{\text{Br}}$  is barely recorded for normal scattering as  $f_{\text{Rb}}$  and  $f_{\text{Br}}$  are almost equal. When  $\text{SrK}\alpha$  is used, the difference becomes appreciable (about two to three electrons) and the 111 and 333 reflections are quite clearly recorded.

The question whether Cu(29) and Mn(25) in the Heusler alloy  $\text{Cu}_2\text{MnAl}$  ( $\text{Fe}_3\text{Al}$  type of structure) have an ordered or disordered arrangement among the crystallographically equivalent Fe positions was difficult to decide under normal X-ray scattering, because of the small difference in the scattering powers of Cu and Mn. The presence of superlattice lines would indicate an ordered structure. But their presence is difficult to detect, as their intensity is extremely small, depending on  $f_{\text{Cu}} - f_{\text{Mn}}$ . By comparative photometric measurement of the powder photographs of  $\text{Cu}_2\text{MnAl}$  with  $\text{CuK}\alpha$  (normal) and  $\text{FeK}\alpha$  (anomalously scattered by Mn,  $\Delta f' = -4.0$ ), Bradley and Rodgers (1934) showed the presence of the superlattice lines, thus establishing the ordered arrangement of Cu and Mn in  $\text{Cu}_2\text{MnAl}$ .

In a similar experiment on  $\beta$ -brass by Jones and Sykes (1937), using

$ZnK\alpha$ , which Cu scatters anomalously ( $\Delta f' = -4.0$ ), the increased difference in  $f_{Cu}$  and  $f_{Zn}$  rendered the superlattice lines sufficiently intense to be clearly recorded.

## 2. Anomalous scattering (without phase change) in determining centrosymmetric structures

(a) *Perfect isomorphism* To use a wavelength anomalously scattered by one set of atoms in a structure is effectively equivalent to substituting that set of atoms by another of different scattering power, at the same atomic sites. The situation is equivalent to a pair of *perfectly* isomorphous structures in normal X-ray scattering. All procedures applicable to a pair of isomorphous crystals, for example isomorphous difference-Patterson, etc., would be valid in this case. Though this had long been realized (see Lipson and Cochran, 1953, p. 227), this was shown to be practicable when Ramaseshan *et al.* (1957) solved the structure of  $KMnO_4$  by anomalous scattering. The special features of this technique are illustrated by examples from this work.

The method uses the small observed differences of the intensity of reflections under normal and anomalous scattering and, as any avoidable inaccuracy will mask or alter the relative differences, success depends greatly on the following steps: (1) Choice of suitable radiations for a given crystal; (2) obtaining satisfactory Weissenberg photographs; (3) proper corrections for geometrical and physical factors and (4) reducing intensity data for the different radiations to the same scale.

(b) *Choice of radiation; scattering factor curve* It would be most advantageous to use two radiations for which the anomalously scattering atom shows the maximum difference in scattering power. If the relatively smaller effects due to the excitation of the  $L$  electrons were neglected,  $\Delta f'$  and  $\Delta f''$  may be read from the curve (Fig. 2), knowing  $\lambda_{K\alpha}/\lambda_{K \text{ edge}}$  for the anomalous scatterer. The best method would be to calculate the scattering factors by the method of Parratt and Hempstead (1954).

In  $KMnO_4$ , where  $CuK\alpha$  and  $FeK\alpha$  were used, the changes  $\Delta f'$  and  $\Delta f''$  for Mn as read from Hönl's curve (1933) are given in Table III. In

TABLE III

$\Delta f'$  and  $\Delta f''$  of manganese from Hönl's (1933) dispersion curve for  $CuK\alpha$  and  $FeK\alpha$

Radiation	$\lambda_i/\lambda_{K \text{ edge}}$	$\Delta f'$	$\Delta f''$
$CuK\alpha$	0.813	-0.78	2.63
$FeK\alpha$	1.021	-4.21	0.00

centrosymmetric structures; the altered scattering factor for any  $(\sin \theta)/\lambda$  may be found by simple vector addition of the normal scattering factor with  $\Delta f'$  and  $\Delta f''$ , assuming these to be independent of angle of scattering. Table IV gives these values for Mn for  $\text{CuK}\alpha$  and  $\text{FeK}\alpha$ .

TABLE IV

The variation of atomic scattering factor of Mn for  $\text{CuK}\alpha$  and  $\text{FeK}\alpha$  with  $(\sin \theta)/\lambda$

$(\sin \theta)/\lambda$	0.0	0.1	0.2	0.3	0.4	0.5	0.6
$f_{\text{CuK}\alpha}$	24.4	21.5	17.6	14.4	12.2	10.6	9.3
$f_{\text{FeK}\alpha}$	20.2	17.9	15.9	10.7	8.5	6.9	5.5

The rapid increase in the ratio of the scattering factors for the two radiations with  $\theta$ , even for small values of  $(\sin \theta)/\lambda$  explains why this technique is practicable even with photographic method of intensity measurement.

(c) *Absorption correction* A reflection  $hkl$  from the crystal occurs in different directions for the two radiations. In a crystal of irregular shape this would mean that different paths are traversed inside the crystal. Further, the two radiations are absorbed differently by the crystal. Any error in the absorption correction would lead to large errors in intensity.

It was found most satisfactory to use a nearly perfect cylindrical crystal of  $\text{KMnO}_4$  and apply absorption corrections for cylindrical specimens.

(d) *Fluorescent radiation and estimation of intensity* If fluorescence is present, as in  $\text{KMnO}_4$  with  $\text{CuK}\alpha$  radiation, and the photographic method is used, the first film in the stack serves as a very efficient filter and intensity measurement may be made with the rest of the films in the stack. One must also bear in mind the variation of the film-to-film intensity ratio, depending on the wavelength used.

(e) *Scale factor* As the method depends on *differences*, it is important to reduce the intensity data with the two radiations to the same relative scale. If not it will lead, for example, in an "anomalous difference-Patterson" (see below) to unwanted background and spurious peaks. A modified form of Wilson's method leads to the following expression for the relative scale factor  $C$  between the two data:

$$\frac{C\langle I \rangle_\lambda}{\langle I \rangle_{\lambda_0}} = \frac{[\sum f_j^2]_\lambda}{[\sum f_j^2]_{\lambda_0}}$$

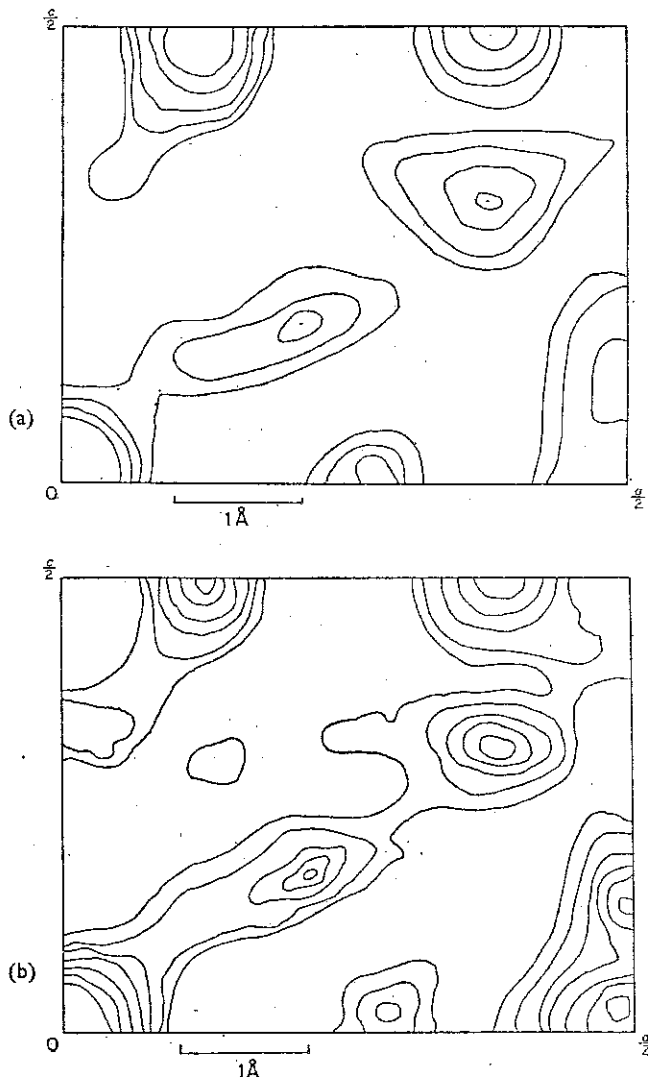


FIG. 11. (a) The  $b$ -axis anomalous difference Patterson projection of  $\text{KMnO}_4$  with  $(|F_{\text{Cu}\kappa\alpha}|^2 - |F_{\text{Fe}\kappa\alpha}|^2)_{h0l}$  as coefficients. (b) The  $b$ -axis isomorphous difference-Patterson with  $(|F_{\text{KMnO}_4}|^2 - |F_{\text{KClO}_4}|^2)_{h0l}$  as coefficients. (Ramaseshan *et al.*, 1957.)

As usual, the averages  $\langle I \rangle$  are taken over narrow ranges of  $(\sin \theta)/\lambda$  to allow for the variation of scattering factors and thermal correction with  $(\sin \theta)/\lambda$ . The procedure proved quite satisfactory in the structure analysis of  $\text{KMnO}_4$ .

(f) *Anomalous difference-Patterson* When intensity data of two isomorphous crystals  $X$  and  $Y$  are available for a wavelength  $\lambda$ , a Patterson

synthesis with  $(F_x^2 - F_y^2)_{hkl}$  as coefficients is the isomorphous difference-Patterson (I.D.P.) (Buerger, 1942b). (In the case of anomalous scattering the analogue of the I.D.P. is the  $(F_\lambda^2 - F_{\lambda_A}^2)$  synthesis which may be called the anomalous difference-Patterson (A.D.P.) synthesis.) The coefficients of this synthesis are the differences for each reflection  $hkl$  of the intensities for  $\lambda$  (normally scattered by all atoms of a structure) and  $\lambda_A$  (anomalously scattered by the atoms  $A$ , and normally scattered by the rest of the structure). The A.D.P. will consist of peaks involving atoms whose scattering powers are altered in anomalous scattering, i.e. (a) interactions among the anomalously scattering atoms themselves and (b) interactions between the anomalously scattering atoms and the normal scatterers. The peaks corresponding to interactions between normal scatterers cancel out in the A.D.P. provided the scaling is proper. The striking similarity between the I.D.P. synthesized with  $(F_{\text{KMnO}_4}^2 - F_{\text{KClO}_4}^2)_{h0l}$  using  $\text{CuK}\alpha$  radiation and the A.D.P. with  $(F_{\text{CuK}\alpha}^2 - F_{\text{FeK}\alpha}^2)_{h0l}$  for  $\text{KMnO}_4$  (Fig. 11(a), (b)) shows that this technique can be successfully used.

(g) *Determination of the position of the anomalous scatterer* A Harker section of the A.D.P. may be used to fix the position of the excited atoms, as this will contain, except for accidental peaks, only the interactions between symmetry equivalent anomalous scatterers.

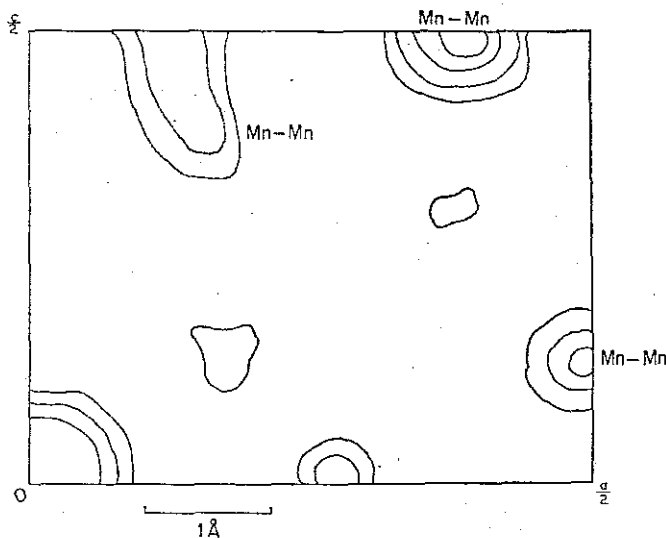


FIG. 12. The  $b$ -axis Patterson projection made with  $(|F_{\text{CuK}\alpha}| - |F_{\text{FeK}\alpha}|)_{h0l}^2$  as coefficients to obtain the position of the anomalously scattering atom in  $\text{KMnO}_4$ . (Ramaseshan *et al.*, 1957.)

A Patterson synthesis with  $(|F_\lambda| - |F_{\lambda_A}|)_{hkl}^2$  as coefficients will, however, be more direct. Now

$$(F_\lambda - F_{\lambda_A})_{hkl} = \sum_A (f_\lambda^A - f_{\lambda_A}^A) \cos 2\pi(hx_A + ky_A + lz_A)$$

and involves only the anomalously scattering atoms  $A$ . Since for most reflections  $(|F_\lambda| - |F_{\lambda_A}|) = F_\lambda - F_{\lambda_A}$ , the  $(|F_\lambda| - |F_{\lambda_A}|)^2$  Patterson is expected to contain only the interactions between the anomalous scatters.

The  $(|F_{CuK\alpha}| - |F_{FeK\alpha}|)^2$  synthesis for  $KMnO_4$  (Fig. 12) shows prominently only the Mn-Mn interaction, as expected.

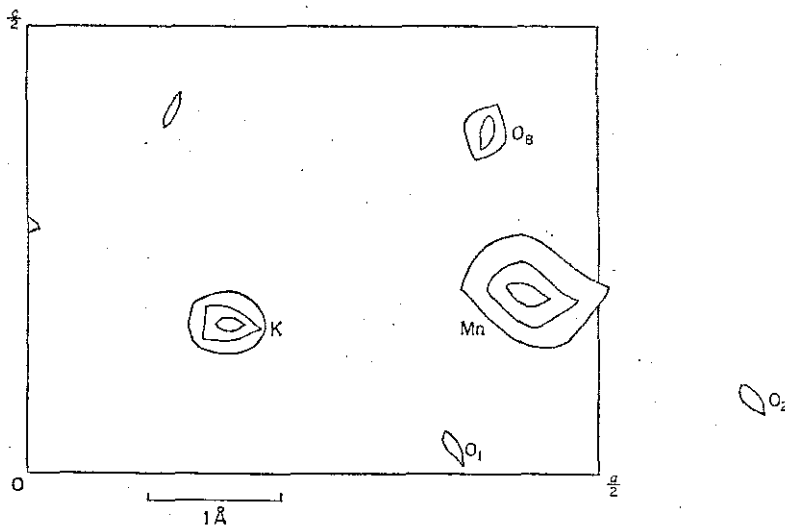


FIG. 13. The structure of  $KMnO_4$  derived from the anomalous difference-Patterson by Buerger's minimum function method.

(h) *Vector shift methods* If the peaks between the anomalous scatterers are thus found it is an easy step to recover the structure by, say, Buerger's minimum function method. Figure 13 shows the structure recovered in this way by two translations of the A.D.P. of  $KMnO_4$ .

(i) *Direct determination of signs* From the equation

$$\{(\pm F_\lambda) - (\pm F_{\lambda_A})\} = \sum_A (f_\lambda^A - f_{\lambda_A}^A) \cos 2\pi(hx_A + ky_A + lz_A),$$

since all the quantities on the right-hand side are known, it is easy to calculate the signs. Over 50% of the signs of the structure amplitudes in  $KMnO_4$  could be so calculated.

### 3. Anomalous scattering without phase change in non-centrosymmetric structures

(a) *Single anomalous scatterer and two incident wavelengths* The technique of anomalous dispersion without phase change may also be applied to non-centrosymmetric structures (Pepinsky and Okaya, 1956; Ramaseshan and Venkatesan, 1957). As this corresponds in effect to isomorphous replacement, all the procedures developed in connection with isomorphous crystals may be applied here. For example, let a structure ( $RA$ ) contain two sets of atoms  $R$  and  $A$ ,  $A$  denoting one set

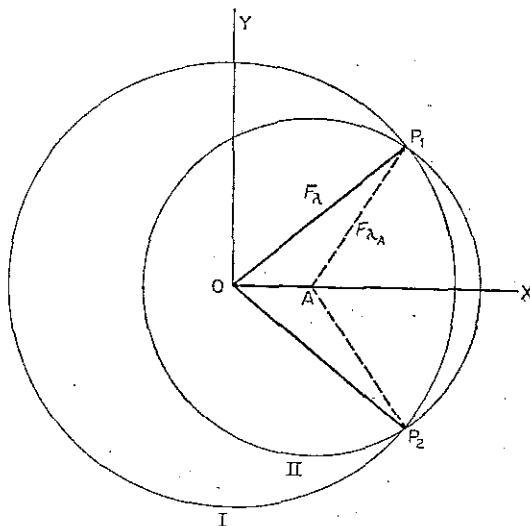


Fig. 14. All the atoms in the structure scatter normally and one set of atoms ( $A$ ) distributed centrosymmetrically scatter  $\lambda_A$  anomalously without phase change.  $F_\lambda$  and  $F_{\lambda_A}$  are the structure amplitudes of a reflection  $hkl$ .  $OA$  is the difference in the scattering factors of  $A$  for  $\lambda$  and  $\lambda_A$ . The figure shows the Harker construction giving the two possible phase angles ( $\angle P_1OX$  and  $\angle P_2OX$ ) for the reflection  $F_\lambda$ .

of atoms which scatter the radiation  $\lambda_A$  anomalously without phase change, and  $R$  the rest of the structure. If intensity data are obtained with  $\lambda_A$  and also with  $\lambda$  (which is scattered normally by both  $R$  and  $A$ ), the situation is similar to the single isomorphous replacement method (Bokhoven *et al.*, 1951). If the anomalously scattering atoms occupy centres of symmetry, i.e.  $\alpha_A = 0$ , the method of arriving at the phases of each reflection by geometrical construction is shown in Fig. 14. There is an ambiguity in the sign of the phase angle. Following Bijvoet, one may perform a synthesis using both the phase angles, which results in a Fourier map with a spurious centre of symmetry.

This map may be interpreted on the basis of knowledge of the chemistry or stereo-chemistry of the molecule under investigation.

Even if the anomalous scatterers do not occupy centres of symmetry, i.e.  $\alpha_A \neq 0$ , the above procedure can be used (Ramachandran and Raman, 1959; Blow and Crick, 1959; Blow and Rossmann, 1961; Ramachandran and Ayyar, 1963). In this two-phased Fourier synthesis, the "true" components add up coherently to give peaks at the atomic sites, while the "error" components give merely a background. The  $\beta_{is}$ -synthesis of Ramachandran is in effect similar to this and is expounded in detail in an article by him in this volume.

(b) *Unique solution* Two different groups of anomalous scatterers and three radiations.

Let a structure  $RAB$  contain two different sets of atoms  $A$  and

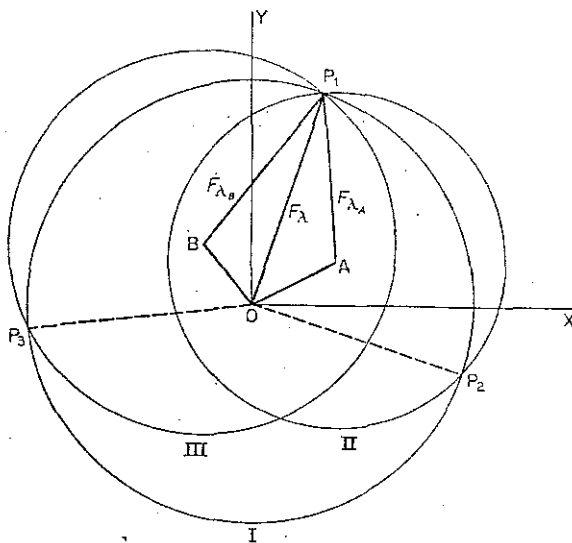


FIG. 15. The crystal consists of three sets of atoms  $R$ ,  $A$  and  $B$ .  $\lambda_A$  and  $\lambda_B$  are scattered anomalously without phase change by  $A$  and  $B$  while  $\lambda$  is scattered normally by all atoms. Figure shows how a unique solution of the phase is obtained.

$B$  which scatter  $\lambda_A$  and  $\lambda_B$  anomalously without phase change. If intensity data are obtained for  $\lambda$  (which is scattered normally by all the atoms),  $\lambda_A$  and  $\lambda_B$ , then the phase of each reflection may be uniquely determined provided the positions of  $A$  and  $B$  are known (Fig. 15). This corresponds to double isomorphous replacement (Harker, 1956). The positions of anomalous scatterers may be determined by making the  $[|F_{\lambda}^{RAB}| - |F_{\lambda_A}^{RAB}|]^2$  and  $[|F_{\lambda}^{RAB}| - |F_{\lambda_B}^{RAB}|]^2$  syntheses.



## B. THE USE OF ANOMALOUS SCATTERING WITH PHASE CHANGE

## 1. Determination of phase angles

Besides its use in determining absolute configuration, Bijvoet and his collaborators showed also how the difference  $|F_{hkl}|^2 - |F_{\bar{h}\bar{k}\bar{l}}|^2$ , which is not equal to zero when anomalous scattering occurs with phase change (i.e.  $\Delta f'' \neq 0$ ), may be used to determine phase angles of X-ray reflections from non-centrosymmetric crystals. A similar method was proposed by Ramachandran and Raman (1956).

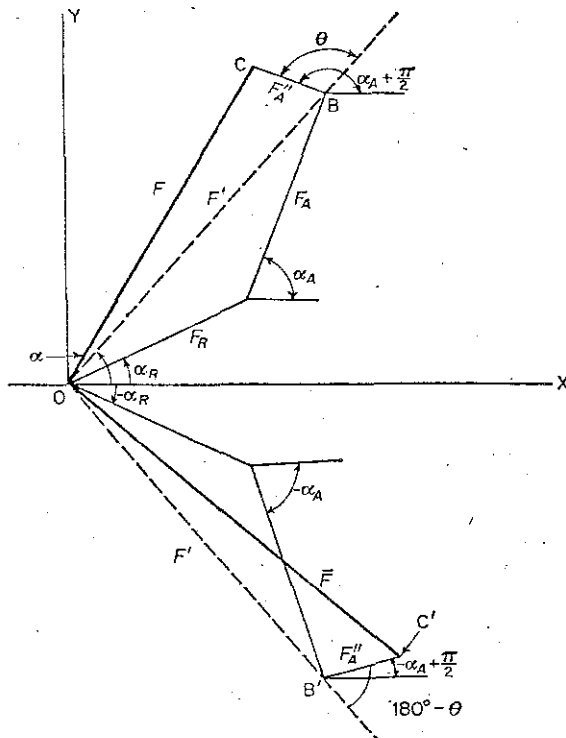


FIG. 16. Determination of the phase of scattering when there is anomalous scattering with phase change.

If  $A$  represents one equivalent set of anomalous scatterers that scatter  $\lambda$  anomalously with phase change, and  $R$  the rest of the structure scattering normally, the structure amplitudes of any reflection  $hkl$  and  $\bar{h}\bar{k}\bar{l}$  (see Fig. 16) are

$$\begin{aligned}
 F = F(hkl) = & \sum_R f_R \exp 2\pi i(hx_R + ky_R + lz_R) + \\
 & + \sum_A (f_{A0} + \Delta f') \exp 2\pi i(hx_A + ky_A + lz_A) + \\
 & + i \sum_A \Delta f'' \exp 2\pi i(hx_A + ky_A + lz_A)
 \end{aligned}$$

$$\begin{aligned}
 &= F_R \exp i\alpha_R + F_A \exp i\alpha_A + iF_A'' \exp i\alpha_A \\
 &= (F_R \exp i\alpha_R + F_A \exp i\alpha_A) + F_A'' \exp i\left(\frac{\pi}{2} + \alpha_A\right) \\
 &= F' \exp i\alpha + F_A'' \exp i\left(\frac{\pi}{2} + \alpha_A\right) \\
 \bar{F} = F(\bar{h}\bar{k}\bar{l}) &= F' \exp -i\alpha + F_A'' \exp i\left(\frac{\pi}{2} - \alpha_A\right)
 \end{aligned}$$

If  $\theta$  is the angle between  $F'$  and  $F_A''$ , the angle between the corresponding vectors for  $\bar{F}$  is  $180 - \theta$ . From  $\triangle OBC$  and  $\triangle OB'C$

$$|F|^2 = |F'|^2 + |F_A''|^2 + 2|F'| |F_A''| \cos \theta$$

$$|\bar{F}|^2 = |F'|^2 + |F_A''|^2 - 2|F'| |F_A''| \cos \theta$$

and

$$\cos \theta = \frac{|F|^2 - |\bar{F}|^2}{4|F'| |F_A''|}$$

$$\theta = \pm \cos^{-1} \left[ \frac{|F|^2 - |\bar{F}|^2}{4|F'| |F_A''|} \right]$$

From the figure it is easily seen that  $\alpha$ , the phase of  $F'$ , is  $\alpha_A + \frac{\pi}{2} \pm \theta$ .

If the positions of the anomalous scatterers are known, then  $\alpha_A$ ,  $|F_A''|$  can be computed and hence  $\theta$  may be obtained from the above equation.

When the anomalous scatterers are centrosymmetrically arranged among themselves in a non-centrosymmetric structure  $\alpha_A = 0$  or  $\pi$  and  $\alpha = \alpha$  or  $\pi - \alpha$ .

## 2. The resolution of the ambiguity

(a) "Heavy atom" method; experimental illustration When the anomalous scatterer is "relatively heavy" in comparison with the rest of the structure, the ambiguity in the phase angle may be resolved by choosing that phase angle which is closer to the phase of the anomalous scatterer. In the structure of ephedrine hydrochloride (Phillips, 1954), Ramachandran and Raman (1956) verified this hypothesis by showing that in over 75% of reflections, the correct phase angle was the one closer to the phase of the anomalous scatterer. This method was first applied for solving the structure of lysine hydrochloride by Raman (1959).

However, the solution of the structure of the compound "factor V1a" ( $C_{46}H_{66}O_9N_{11}Co$ , 11  $H_2O$ ), recently by Dale, Hodgkin and Venkatesan (1963) is perhaps the most striking proof of the power of the anomalous scattering technique. The intensity data were collected only by photographic methods, using  $CuK\alpha$  which is scattered anomalously by cobalt with  $\Delta f' = -2.2$  and  $\Delta f'' = 3.9$ . The dispersion effects were very

marked, and the breakdown of Friedel's law was apparent even visually. The technique of anomalous scattering with phase change enabled these authors to calculate from the intensities of Bijvoet pairs the phases of 1994 out of nearly 4000 observed reflections. The anomalous scatterers Co in this compound were centrosymmetrically disposed so that  $\alpha_A = 0$ . Where the reflections of a pair appeared on opposite sides of the centre line in Weissenberg photographs correction for extension and contraction of spots was also necessary. Instead of Phillips's (1956) correction, the authors were content to correct for this by the equations

$$I_{\text{true}} = I_e(1 + k \cos \theta)$$

$$I_{\text{true}} = I_c(1 - k \cos \theta)$$

where the subscripts  $e$  and  $c$  stand for extended and contracted spots respectively, and  $\theta$  is the Bragg angle. This correction presumably was adequate.

Since the temperature factor affects the observed structure amplitudes,  $F'_A$  must be multiplied by a suitable temperature factor, which may be obtained from Wilson's scale factor curve. Nor must it be forgotten during structure factor computation that  $\Delta f' \neq 0$ . Neglect of this may be compensated for during structure analysis only by a wrong temperature factor which may affect the accuracy of the atomic parameters.

The technique using anomalous scattering with phase change automatically leads to the absolute configuration.

(b) *The two-phased synthesis* Ramachandran and Raman (1956) suggested where the anomalous scatterers were themselves centrosymmetrically arranged in a non-centric structure, a Fourier synthesis might be made using both phases, i.e. using for each reflection in the synthesis both  $(|F|, \alpha)$  and  $(|F|, \pi - \alpha)$ . The procedure is similar to Bijvoet's method (see Bokhoven *et al.*, 1951) for a pair of isomorphous structures under normal scattering, except that the map here contains a structure and its "negative inverse", i.e. for a peak at  $xyz$  there is at  $\bar{x}\bar{y}\bar{z}$  a negative peak of the same magnitude. Apparently such a map should be easier to deal with than when it contains a structure and its inverse. One must be careful in interpreting such a map for complex structures when the peak belonging to a structure may be wiped out because it coincides with the negative inverse of another peak.

The method may be used even when the anomalous scatterers are not centrosymmetrically disposed, i.e. a Fourier synthesis may be made with both

$$\left\{ |F|, \left( \alpha_A + \frac{\pi}{2} + \theta \right) \right\} \quad \text{and} \quad \left\{ |F|, \left( \alpha_A + \frac{\pi}{2} - \theta \right) \right\}$$

The correct terms will reinforce each other at the correct atomic positions and the incorrect ones cancel each other and give merely a background. The convergence to a correct structure will, however, be slow.

(c) *Unique solutions; the isomorphous replacement method and the two-wavelengths method* The inherent ambiguity in the phases

$$\alpha = \alpha_A + \frac{\pi}{2} \pm \theta$$

when the technique of anomalous scattering with phase change is used, can be resolved if this is combined with the isomorphous replacement method (Peerdeman *et al.*, 1951). The method consists in measuring the intensities of  $I^{RA}(hkl)$  and  $I^{RA}(\bar{h}\bar{k}\bar{l})$  which are different for a radiation  $\lambda_A$  which is scattered anomalously by set of atoms  $A$  in the structure, and  $I^{RB}(hkl)$  for an isomorphous crystal. By using the Harker construction and knowing the positions of the replaceable atoms, the phases can be determined uniquely.

Anomalous scattering without phase change is equivalent to isomorphous replacement. Hence, for the same crystal  $RA$ , if intensity data are collected for  $\lambda'_A$ , which is scattered anomalously without phase change by the atoms  $A$ , the ambiguity can be resolved. How far these small differences in intensities may actually be used for the solution of the phase problem in this manner is a matter of conjecture. However, when proper weighting functions are used (Blow, 1958; Blow and Crick, 1959; Blow and Rossmann, 1961), it is spectacular to note how a large number of terms, each of which by itself is of little significance, can result in important cumulative effects, when combined in a Fourier transform.

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