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# The Determination of the Phases of the Structure Factors of Non-Centrosymmetric Crystals by the Method of Double Isomorphous Replacement*

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It is shown that the complete structure of a non-centrosymmetric crystal can be determined from the X-ray diffraction data obtained from the members of an isomorphous series in which two different parts of the structure can be varied independently. The structure so determined may be an enantiomorph of the true structure.

## 1. Introduction

Two crystals are said to be isomorphous if they have essentially the same structures, but are composed of chemically different atoms. (This includes the case of additional atoms, since these can be thought of as replacing vacancies, i.e. atoms of zero atomic number.) Examples of isomorphism are very common: $KMnO_4$ and $BaSO_4$, $Ag_3AsS_3$ and $Ag_3SbS_3$, benzene hexachloride and benzene hexabromide, tetraphenyl tin and tetraphenyl lead, etc. It often happens that the several hydrohalides of a complicated organic amine, or the various alkali metal salts of an organic acid, form series of isomorphous crystals. It has recently been discovered that certain proteins can crystallize in apparently identical fashion either with or without the substitution of mercury or silver atoms for the hydrogen of sulfhydryl groups, or with or without dye molecules bound to the protein molecules; these are also examples of isomorphism. The existence of such isomorphous pairs or series often greatly facilitates the determination of the structure of these crystals by X-ray diffraction methods.

A good account of how isomorphous replacement methods have been used for determining the structures of crystals is given in the new book by Lipson & Cochran (1953). These methods have been applied in the past with great success to centrosymmetric crystals.

It is often important to find the structure of a complicated organic molecule containing dozens, or even hundreds, of light atoms. This can be done when a crystal containing this molecule is capable of forming two different isomorphous series by the addition or substitution of heavy atoms in two different sets of positions in the unit cell. For example, suppose that a certain complicated sugar forms a set of isomorphous crystalline addition compounds with the alkali halides NaCl, NaBr and KCl. The intensities of the X-rays diffracted by these three crystals can be used to define completely the structure of the sugar, as will be shown below, provided only that the arrangements of the alkali and halide atoms are sufficiently simple that their positions can be determined easily (see, however, the discussion in § 3). This latter problem, that of finding the positions of the heavy atoms, is usually soluble by the use of Patterson functions or related methods; in the discussion to follow, it is assumed that the arrangements of the heavy atoms have been found, and that the question of interest concerns the nature of the complicated arrangements of the light atoms in the crystal.

Bokhoven, Schoone & Bijvoet (1951; see especially the first full paragraph on p. 279) have outlined very briefly the method of double isomorphous replacement which is the subject of the present paper. They did not, however, mention the difficulties connected with the choice of origin and enantiomorphism, which are discussed at some length below in § 5. The resolution of these ambiguities is imperative for the solution of a non-centrosymmetric structure. The detailed development of the entire method has therefore been undertaken here.

## 2. Statement and solution of the problem

The structure factors, $F(hkl)$, of a crystal depend on the natures and positions of the atoms in one unit cell of the crystal according to the formula:

$$F(hkl) = \sum_{j=1}^{N} f_j(hkl) \exp 2\pi i(hx_j+ky_j+lz_j) , \qquad (1)$$

where the various symbols have their usual meanings. The sum on the right of (1) can be conveniently divided into terms corresponding to the atoms which do not change from one isomorphous crystal to another and other terms which correspond to atoms which do change. Call the constant part of the structure $O$ and the variable part of the structure $X$. Then

$$F_{O+X} = F_O+F_X . \qquad (2)$$

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The indices $h$, $k$ and $l$ have been omitted here, since no confusion is possible, and the subscripts indicate the parts of the structure to which each term corresponds. Inasmuch as $F(hkl)$ is, in general, a complex number, equation (2) is a vector equation in the complex plane, and can be represented by a diagram such as Fig. 1.

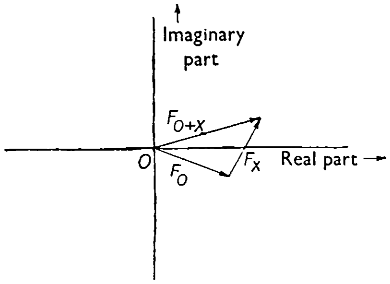


Fig. 1. Vector addition of the structure factors of the invariable and variable parts of a crystal structure.

It is assumed in what follows that the experimental quantities $|F(hkl)|$ are known for the various members of the isomorphous series. For definiteness, assume that, for each $h, k, l$, the magnitudes $|F_O|$, $|F_{O+X}|$ and $|F_{O+M}|$ are known, where $X$ and $M$ refer to two different isomorphous additions to the structure $O$. Assume also that the structures $X$ and $M$ have been found, and that the complex numbers $F_X$ and $F_M$ have been computed from them for each $h, k, l$. The problem is to find the complex numbers $F_O(hkl)$ from which the structure $O$ can be computed directly by Fourier methods.

The solution for $F_O$ is easily obtained graphically; the procedure is illustrated in Fig. 2. Vectors $-F_X$

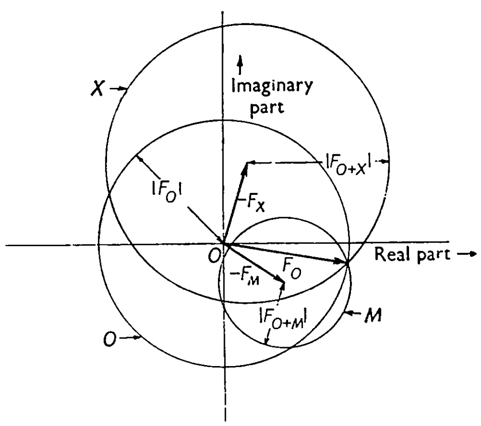


Fig. 2. Solution of the vector equations for $F_O$ from a knowledge of $|F_O|$, $|F_{O+M}|$, $|F_{O+X}|$, $F_M$ and $F_X$.

and $-F_M$ are drawn from the origin of the complex plane, and a circle (marked $O$) with radius equal to $|F_O|$ is described about the origin as center, a circle (marked $X$) of radius $|F_{O+X}|$ is centered at the end of the vector $-F_X$ and another circle (marked $M$) of radius $|F_{O+M}|$ is centered at the end of vector $-F_M$. The three circles intersect at a point which is the end of vector $F_O$. It is to be noted that a knowledge of $F_X$, $|F_O|$ and $|F_{O+X}|$ alone gives two solutions for $F_O$, one for each of the two intersections of circles $O$ and $X$; similarly, there are two solutions for $F_O$ obtainable from a knowledge of $F_M$, $|F_O|$ and $|F_{O+M}|$ alone, corresponding to the two intersections of circles $O$ and $M$. The correct solution for $F_O$ is the one common to these pairs of solutions, i.e. the one corresponding to the point where all three circles $O$, $M$ and $X$ intersect.

Analytically, each complex number $F$ can be written $F = A+iB$. The known data are $|F_O|^2 = A_O^2+B_O^2$, $|F_{O+X}|^2 = A_{O+X}^2+B_{O+X}^2$, $|F_{O+M}|^2 = A_{O+M}^2+B_{O+M}^2$, $F_X = A_X+iB_X$ and $F_M = A_M+iB_M$. We wish to find $A_O$ and $B_O$. Now,

$$\begin{aligned}|F_{O+X}|^2 &= (A_O+A_X)^2+(B_O+B_X)^2 \\ &= A_O^2+B_O^2+A_X^2+B_X^2+2A_OA_X+2B_OB_X\end{aligned}$$

or

$$|F_{O+X}|^2 = |F_O|^2+|F_X|^2+2A_OA_X+2B_OB_X\,.$$

This gives:

$$P_X = |F_{O+X}|^2-|F_X|^2-|F_O|^2 = 2A_XA_O+2B_XB_O\,. \quad (3)$$

Similarly,

$$P_M = |F_{O+M}|^2-|F_M|^2-|F_O|^2 = 2A_MA_O+2B_MB_O\,. \quad (4)$$

All the quantities in (3) and (4) are known, except $A_O$ and $B_O$; consequently these two equations can be solved simultaneously, as follows:

Let

$$\Delta = 4(A_XB_M-A_MB_X)\,,$$

then

$$A_O = \frac{2}{\Delta}\begin{vmatrix} P_X & B_X \\ P_M & B_M \end{vmatrix} \quad \text{and} \quad B_O = \frac{2}{\Delta}\begin{vmatrix} A_X & P_X \\ A_M & P_M \end{vmatrix}. \quad (5)$$

From the values of $A_O$ and $B_O$ obtained by means of (5) it is possible to write immediately $F_O = A_O+iB_O$.

The analytical method just outlined corresponds to locating the end of the vector $F_O$ from a knowledge of its projections on the directions of the two vectors $F_M$ and $F_X$. The geometry is thus quite different from that of the most convenient graphical method, and the effect of experimental errors in the intensity measurements will not be the same. The analytical method always gives a unique determination of $A_O$ and $B_O$ (except when $F_M$ and $F_X$ are parallel), while the graphical method may not, since there may be no point at which the three circles $O$, $M$ and $X$ intersect. If the graphical method fails in this way, the best position for the end of the vector $F_O$ would be in the center of the small curved triangle where the three circles nearly intersect; an averaging of errors is also required in the analytic method, because $A_O^2+B_O^2$ may differ from the observed value of $|F_O|^2$, but the averaging is different.

## 3. Situations to be avoided

It is easy to see that, if $F_M$ and $F_X$ are parallel or antiparallel (i.e. collinear in Fig. 2), there will be two points where the three circles $O$, $M$ and $X$ intersect, corresponding to two solutions for $F_O$. In this case the analytical method just described fails to give a unique solution for $F_O$, since $\Delta = 0$. This situation may occur occasionally in any one isomorphous series for special values of $h, k, l$, but such a sporadic ambiguity usually does not seriously hamper a structure determination. If, on the other hand, $F_M$ and $F_X$ are systematically collinear, no structure determination by this method is possible for a non-centrosymmetric crystal. For instance, this last is true for every $h, k, l$, if structures $M$ and $X$ are both centrosymmetric about the same point in structure $O$, or centrosymmetric about points which differ in coordinates by integral numbers of halves; and there are several other special situations which make $F_M$ and $F_O$ collinear for a hopelessly large proportion of the $h, k, l$, combinations. Such situations cannot occur if one of the structures $M$ or $X$ is non-centrosymmetric, or if they are both centrosymmetric, but about points which differ in coordinates by irrational numbers. Thus, in the case of the series of alkali halide–sugar addition compounds mentioned in § 1, if each unit cell contains only one alkali metal atom and one halogen atom, the coordinates of these must differ by irrational numbers, in order to make the structure determination possible, since single atoms are always centrosymmetric. (Of course, if the crystal is centrosymmetric—a case not under consideration here—the structure determination is usually possible, even though $F_M$ and $F_X$ are required by symmetry to be collinear.)

## 4. Solution of a hypothetical structure

These methods were applied to a hypothetical one-dimensional structure consisting of point atoms, of unit scattering power for X-rays, arranged along a line. All atomic coordinates are multiples of one-twelfth of the period, so that the $F$'s are periodic functions of the order of 'reflection', $h$, with a period of twelve, and the relationship $F(h) = F^*(12n-h)$ holds, where the asterisk indicates the complex conjugate and $n$ is an integer. It is therefore only necessary to study the first six orders of reflection. The structure $O$ was taken to consist of six atoms, while the two different structures $M$ and $X$ were each taken to have three atoms. None of these structures has a center of symmetry. The atomic coordinates appear in Table 1, and the various values of $F$, $|F|$ and $|F|^2$, together with the phase angle $\alpha$, from $F = |F| \exp(i\alpha)$, are presented in Table 2.

A test of the graphical method is illustrated in Fig. 3. For each order of reflection $h$, two diagrams appear in the figure; one shows the determination of the two solutions for $F_O$ found by the use of $F_M$, $|F_{O+M}|$ and $|F_O|$, the other shows the two solutions found by the use of $X$, instead of $M$. Comparing these two diagrams, it is always found that one solution for $F_O$ is common to both and has the same phase angle $\alpha$ as appears in Table 2. It is easy to carry out the computations of the analytical method and to show that it, too, gives the values of $F_O$ in Table 2.

Table 1. *Coordinates of unit point atoms in hypothetical test structures*

Structure $O$:

Atoms at $x = \frac{1}{12}, \frac{2}{12}, \frac{3}{12}, \frac{7}{12}, \frac{10}{12}, \frac{11}{12}$

$$F_O(h) = 2\cos 2\pi\frac{h}{12} + 2\cos 2\pi\frac{h}{6} + (i)^h + (-1)^h \exp\left(2\pi i\frac{h}{12}\right)$$

Structure $M$:

Atoms at $x = 0, \frac{5}{12}, \frac{8}{12}$

$$F_M(h) = 1 + (-1)^h \exp\left(-2\pi i\frac{h}{12}\right) + \exp\left(-2\pi i\frac{h}{3}\right)$$

Structure $X$:

Atoms at $x = \frac{4}{12}, \frac{6}{12}, \frac{9}{12}$

$$F_X(h) = \exp\left(2\pi i\frac{h}{3}\right) + (-1)^h + (-i)^h$$

Structure $O+M$:

Atoms at positions of structures $O$ and $M$

$$F_{O+M}(h) = F_O(h) + F_M(h)$$

Structure $O+X$:

Atoms at positions of structures $O$ and $X$

$$F_{O+X}(h) = F_O(h) + F_X(h)$$

## 5. Ambiguities and their removal

In an actual application of this method of double isomorphous replacement, some complications arise which have not yet been mentioned and which have to do with the indeterminacy of the origin to which a structure is referred and with the near impossibility of distinguishing between two enantiomorphs by X-ray diffraction. These difficulties will be discussed using the structures $O$, $M$ and $X$ of Table 1. Structure $M$, for instance, must be discovered by comparing the Patterson functions of structures $O$ and $O+M$, since a crystal of structure $M$ alone cannot exist. This process results in a knowledge of the interatomic vectors in $M$, and from this it is usually possible to find the relative positions of its atoms, except that either enantiomorph will have the same interatomic vectors and that any origin can be used. Thus, starting from the values of $|F_O|^2$ and $|F_{O+M}|^2$ of Table 2, it could be found that structure $M$ consists of three atoms in a row, the two end ones being, respectively, 3/12 and 4/12 of the period from the middle one, but there would be no way to tell which end should be in the direction of increasing $X$. (This description of structure $M$ corresponds to choosing the origin at $x = 8/12$ and using the atoms at $x = 5/12$, 8/12 and 1 in the descrip-

Table 2. *Values of F, |F|, |F|² and α for the structures O, M, X, O+M and O+X of Table* 1

$(F = |F|e^{i\alpha} = A+iB)$

| $h$ | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| $F_O(h)$ | 6 | $\frac{1}{2}(2+\sqrt{3})+\frac{1}{2}i$ | $-\frac{1}{2}+\frac{1}{2}\sqrt{3}i$ | $-2-2i$ | $-\frac{3}{2}+\frac{1}{2}\sqrt{3}$ | $\frac{1}{2}(2-\sqrt{3})+\frac{1}{2}i$ | $-2$ |
| $\alpha_O(h)$ (°) | 0 | 15 | 120 | 225 | 150 | 75 | 180 |
| $\|F_O(h)\|$ | 6 | $\frac{1}{2}\sqrt{2}(\sqrt{3}+1)$ | 1 | $2\sqrt{2}$ | $\sqrt{3}$ | $\frac{1}{2}\sqrt{2}(\sqrt{3}-1)$ | 2 |
| $\|F_O(h)\|^2$ | 36 | $2+\sqrt{3}$ | 1 | 8 | 3 | $2-\sqrt{3}$ | 4 |
| | | | | | | | |
| $F_M(h)$ | 3 | $-\frac{1}{2}(\sqrt{3}-1)-\frac{1}{2}(\sqrt{3}-1)i$ | 1 | $2+i$ | $-\sqrt{3}i$ | $\frac{1}{2}(\sqrt{3}+1)+\frac{1}{2}(\sqrt{3}+1)i$ | 1 |
| $\alpha_M(h)$ (°) | 0 | 225 | 0 | 26·56 | 270 | 45 | 0 |
| $\|F_M(h)\|$ | 3 | $\frac{1}{2}\sqrt{2}(\sqrt{3}-1)$ | 1 | $\sqrt{5}$ | $\sqrt{3}$ | $\frac{1}{2}\sqrt{2}(3+1)$ | 1 |
| $\|F_M(h)\|^2$ | 9 | $2-\sqrt{3}$ | 1 | 5 | 3 | $2+\sqrt{3}$ | 1 |
| | | | | | | | |
| $F_X(h)$ | 3 | $-\frac{3}{2}-\frac{1}{2}(2-\sqrt{3})i$ | $-\frac{1}{2}-\frac{1}{2}\sqrt{3}i$ | $i$ | $\frac{3}{2}+\frac{1}{2}\sqrt{3}i$ | $-\frac{3}{2}-\frac{1}{2}(2+\sqrt{3})i$ | 1 |
| $\alpha_X(h)$ (°) | 0 | 185·11 | 240 | 90 | 30 | 231·21 | 0 |
| $\|F_X(h)\|$ | 3 | $\sqrt{(4-\sqrt{3})}$ | 1 | 1 | $\sqrt{3}$ | $\sqrt{(4+\sqrt{3})}$ | 1 |
| $\|F_X(h)\|^2$ | 9 | $4-\sqrt{3}$ | 1 | 1 | 3 | $4+\sqrt{3}$ | 1 |
| | | | | | | | |
| $F_{O+M}(h)$ | 9 | $\frac{3}{2}+\frac{1}{2}(2-\sqrt{3})i$ | $\frac{1}{2}+\frac{1}{2}\sqrt{3}i$ | $-i$ | $-\frac{3}{2}-\frac{1}{2}\sqrt{3}i$ | $\frac{3}{2}+\frac{1}{2}(2+\sqrt{3})i$ | $-1$ |
| $\alpha_{O+M}(h)$ (°) | 0 | 5·11 | 60 | 270 | 210 | 51·21 | 180 |
| $\|F_{O+M}(h)\|$ | 9 | $\sqrt{(4-\sqrt{3})}$ | 1 | 1 | $\sqrt{3}$ | $\sqrt{(4+\sqrt{3})}$ | 1 |
| $\|F_{O+M}(h)\|^2$ | 81 | $4-\sqrt{3}$ | 1 | 1 | 3 | $4+\sqrt{3}$ | 1 |
| | | | | | | | |
| $F_{O+X}(h)$ | 9 | $\frac{1}{2}(\sqrt{3}-1)+\frac{1}{2}(\sqrt{3}-1)i$ | $-1$ | $-2-i$ | $\sqrt{3}i$ | $-\frac{1}{2}(\sqrt{3}+1)-\frac{1}{2}(\sqrt{3}+1)i$ | $-1$ |
| $\alpha_{O+X}(h)$ (°) | 0 | 45 | 180 | 206·56 | 90 | 225 | 180 |
| $\|F_{O+X}(h)\|$ | 9 | $\frac{1}{2}\sqrt{2}(\sqrt{3}-1)$ | 1 | $\sqrt{5}$ | $\sqrt{3}$ | $\frac{1}{2}\sqrt{2}(\sqrt{3}+1)$ | 1 |
| $\|F_{O+X}(h)\|^2$ | 81 | $2-\sqrt{3}$ | 1 | 5 | 3 | $2+\sqrt{3}$ | 1 |

Table 3. *Values of F, α, F and F² for structures M′ and X′*

| $h$ | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| $F_{M'}(h)$ | 3 | $\frac{1}{2}+\frac{1}{2}(2-\sqrt{3})i$ | $-\frac{1}{2}+\frac{1}{2}\sqrt{3}i$ | $2-i$ | $\frac{3}{2}-\frac{1}{2}\sqrt{3}i$ | $\frac{1}{2}+\frac{1}{2}(2+\sqrt{3})i$ | 1 |
| $\alpha_{M'}(h)$ (°) | 0 | 15 | 120 | 333·44 | 330 | 75 | 0 |
| $\|F_{M'}(h)\|$ | 3 | $\frac{1}{2}\sqrt{2}(\sqrt{3}-1)$ | 1 | $\sqrt{5}$ | $\sqrt{3}$ | $\frac{1}{2}\sqrt{2}(\sqrt{3}+1)$ | 1 |
| $\|F_{M'}(h)\|^2$ | 9 | $2-\sqrt{3}$ | 1 | 5 | 3 | $2+\sqrt{3}$ | 1 |
| | | | | | | | |
| $F_{X'}(h)$ | 3 | $\frac{3}{2}+\frac{1}{2}(2-\sqrt{3})i$ | $-\frac{1}{2}-\frac{1}{2}\sqrt{3}i$ | $-i$ | $\frac{3}{2}+\frac{1}{2}\sqrt{3}i$ | $\frac{3}{2}+\frac{1}{2}(2+\sqrt{3})i$ | 1 |
| $\alpha_{X'}(h)$ (°) | 0 | 5·11 | 240 | 270 | 30 | 51·21 | 0 |
| $\|F_{X'}(h)\|$ | 3 | $\sqrt{(4-\sqrt{3})}$ | 1 | 1 | $\sqrt{3}$ | $\sqrt{(4+\sqrt{3})}$ | 1 |
| $\|F_{X'}(h)\|^2$ | 9 | $4-\sqrt{3}$ | 1 | 1 | 3 | $4+\sqrt{3}$ | 1 |

tion of Table 1, with $x$ increasing either to the right or left.) It might well be decided to use the coordinates $x = 0$, 3/12, 8/12 for the atoms in $M$, and this would be a 'correct' structure determination, as this phrase is usually used; this structure will be called $M'$. In a similar way, structure $X$ might be 'correctly' determined to have atoms at $x = 0$, 3/12, 10/12; this structure will be called $X'$. It will be noted that structure $M'$ is the enantiomorph of $M$, while structure $X'$ is congruent to $X$; also that the origins of $M'$ and $X'$ are not properly chosen with respect to one another. The values of $F$, $\alpha$, $|F|$ and $|F|^2$ for structures $M'$ and $X'$ appear in Table 3. ($|F|$ and $|F|^2$ are, of course, the same as in Table 2.)

Since structures $M'$ and $X'$ are referred to different origins and correspond to different enantiomorphs, the solution for $F_O$ cannot be obtained directly from the values of $F_{M'}$ and $F_{X'}$ by the use of the methods previously described. However, as will be shown, it is possible to extend these methods so as to obtain a 'correct' set of values for $F_O$, in the sense that these values will correspond to structure $O$ or its enantiomorph and that structure $O$ may be referred to a different origin from that in the original description.

First, consider the effect on the values of $F(hkl)$ caused by changing the origin to which the crystal structure is referred. Suppose the new origin has coordinates $x_0$, $y_0$, $z_0$ in the original coordinate system, and let $F'(hkl)$ be the value of $F(hkl)$ when the new origin is used.

If the complex number $F(hkl)$ is written in the form $F = |F| \exp (i\alpha)$, then the value of $F'(hkl)$ is

$$F'(hkl) = |F(hkl)| \exp i[\alpha(hkl)-2\pi(hx_0+ky_0+lz_0)] \,. \quad (6)$$

This is the same as saying that the magnitudes of the $F$'s are not changed by a change of origin, but the phase angles $\alpha$ (expressed in radians) are reduced by $2\pi(hx_0+ky_0+lz_0)$. For a one-dimensional structure, the reduction in $\alpha$ is $2\pi hx_0$.

Second, the effect of changing from a structure to its enantiomorph is most easily expressed by changing all coordinates $x_j$, $y_j$, $z_j$ into negatives: $-x_j$, $-y_j$, $-z_j$. This clearly changes each $F$ into $F^*$ and therefore changes $\alpha$ into $-\alpha$.

Let us now attempt to find the 'correct' values of $F_O$ for the hypothetical one-dimensional structure of Table 1, starting with the measured values of $|F_O|$,

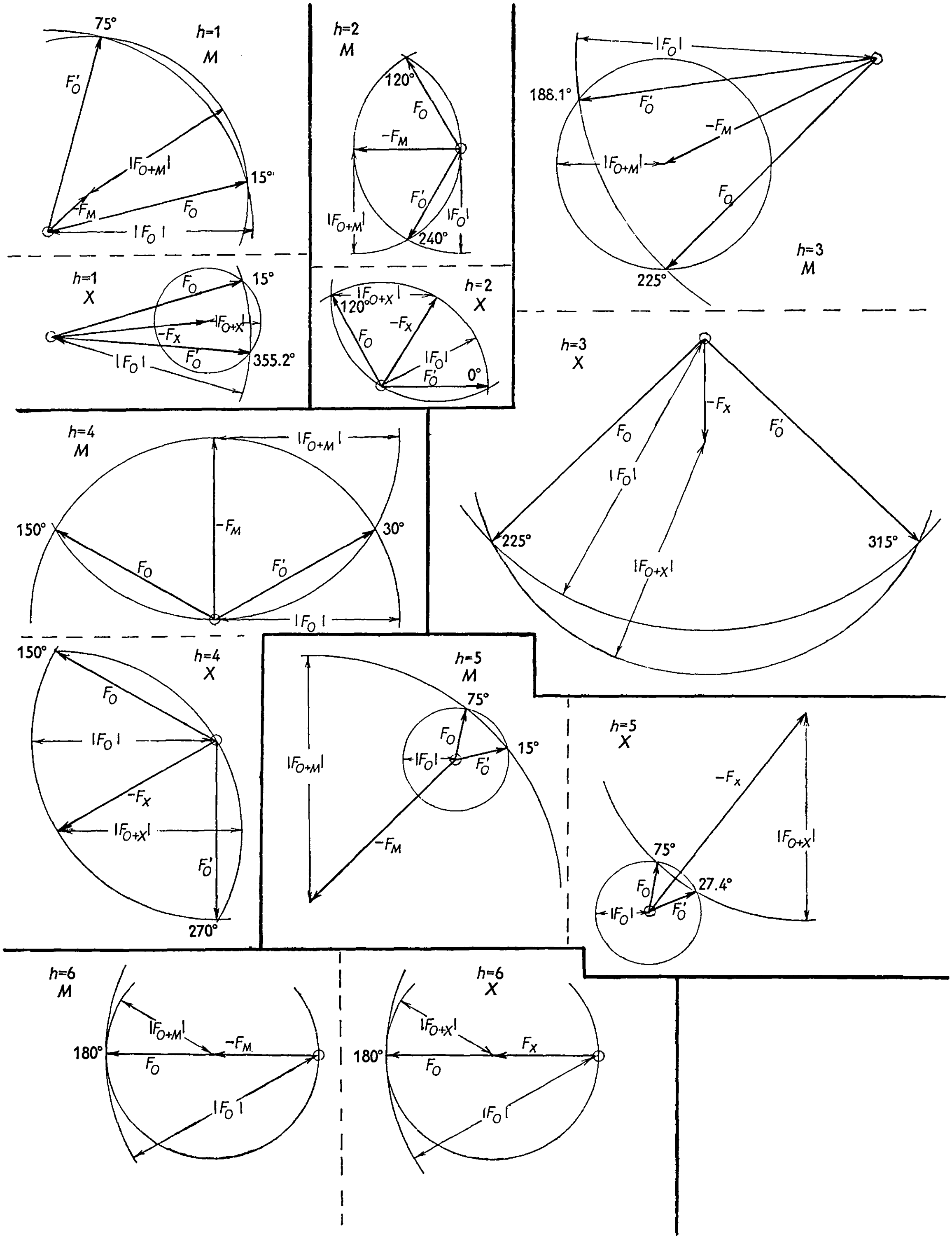


Fig. 3. Test of the double isomorphous replacement method of phase determination.

$|F_{O+M}|$, $|F_{O+X}|$ and the values of $F_{M'}$ and $F_{X'}$ found arbitrarily and listed in Table 3; we shall keep in mind, however, that structures $M'$ and $X'$ may be referred to different origins, and may correspond to different enantiomorphs.

The first step is to find the two values of $\alpha_O(h)$

Table 4. *Values of* $\alpha_O$ *found from* $|F_O|$, $|F_{O+M}|$ *and* $F_{M'}$ *and from* $|F_O|$, $|F_{O+X}|$ *and* $F_{X'}$.

(All values of $\alpha$ are in degrees)

| $h$ | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| | | | (*a*) | | | | |
| $\|\alpha_O-\alpha_M\|$ | 0 | 150 | 120 | 198·44 | 240 | 30 | 180 |
| $\alpha_{M'}$ | 0 | 15 | 120 | 333·44 | 330 | 75 | 0 |
| $\alpha_{M'}+\|\alpha_O-\alpha_{M'}\| = \alpha_O'$ | 0 | 165 | 240 | 171·88 | 210 | 105 | 180 |
| $\alpha_{M'}-\|\alpha_O-\alpha_{M'}\| = \alpha_O''$ | 0 | 225 | 0 | 135 | 90 | 45 | 180 |
| | | | (*b*) | | | | |
| $\|\alpha_O-\alpha_X\|$ | 0 | 189·89 | 240 | 135 | 120 | 203·79 | 180 |
| $\alpha_{X'}$ | 0 | 5·11 | 240 | 270 | 30 | 51·21 | 0 |
| $\alpha_X+\|\alpha_O-\alpha_{X'}\| = \alpha_O'''$ | 0 | 195 | 120 | 45 | 150 | 255 | 180 |
| $\alpha_{X'}-\|\alpha_O-\alpha_{X'}\| = \alpha_O^{iv}$ | 0 | 175·22 | 0 | 135 | 270 | 207·42 | 180 |
| | | | (*c*) | | | | |
| $\alpha_O'-\alpha_O'''$ | 0 | 330 | 120 | 126·88 | 60 | 210 | 0 |
| $\alpha_O'-\alpha_O^{iv}$ | 0 | 349·78 | 240 | 36·88 | 300 | 257·58 | 0 |
| $\alpha_O''-\alpha_O'''$ | 0 | 30 | 240 | 90 | 300 | 150 | 0 |
| $\alpha_O''-\alpha_O^{iv}$ | 0 | 49·78 | 0 | 0 | 180 | 197·58 | 0 |
| | | | (*d*) | | | | |
| $\alpha_O'+\alpha_O'''$ | 0 | 0 | 0 | 216·88 | 0 | 0 | 0 |
| $\alpha_O'+\alpha_O^{iv}$ | 0 | 340·22 | 240 | 306·88 | 120 | 312·42 | 0 |
| $\alpha_O''+\alpha_O'''$ | 0 | 60 | 120 | 180 | 240 | 300 | 0 |
| $\alpha_O''+\alpha_O^{iv}$ | 0 | 40·22 | 0 | 270 | 0 | 252·42 | 0 |
| | | | (*e*) | | | | |
| $F_{X'''}(h)$ | 3 | $\frac{1}{2}\sqrt{3}+\frac{1}{2}(2\sqrt{3}-1)i$ | $-\frac{1}{2}-\frac{1}{2}\sqrt{3}i$ | $-i$ | $-\frac{3}{2}-\frac{1}{2}\sqrt{3}i$ | $-\frac{1}{2}\sqrt{3}-\frac{1}{2}(2\sqrt{3}+1)i$ | 1 |
| $\alpha_{X'''}$ | 0 | 54·89 | 240 | 270 | 210 | 248·79 | 0 |
| $\|\alpha_O-\alpha_{X'''}\|$ | 0 | 189·89 | 240 | 135 | 120 | 203·79 | 180 |
| $\alpha_{X'''}+\|\alpha_O-\alpha_{X'''}\| = \alpha_O^{v}$ | 0 | 244·78 | 120 | 45 | 330 | 42·58 | 180 |
| $\alpha_{X'''}-\|\alpha_O-\alpha_{X'''}\| = \alpha_O^{vi}$ | 0 | 225 | 0 | 135 | 90 | 45 | 180 |

that correspond to each combination $|F_O|$, $|F_{O+M}|$, $F_{M'}$ and $|F_O|$, $|F_{O+X}|$, $F_{X'}$. This can be done either graphically, as in Fig. 3, or analytically. It is easily shown that:

$$\cos(\alpha_O-\alpha_M) = \frac{1}{2|F_O|\,|F_M|}[|F_{O+M}|^2-|F_M|^2-|F_O|^2], \quad (7)$$

where $\alpha_O$ and $\alpha_M$ are the phase angles of $F_O$ and $F_M$, respectively. From (7), the magnitude of $\alpha_O-\alpha_M$ can be found, but not its sign; if $\alpha_M$ is known, this gives two values of $\alpha_O$—the same two values to be found by the graphical method. Formula (7) applies equally well, of course, if $M$ is everywhere replaced by $X$. Table 4 presents the results of calculations based on this method.

In Table 4 (*a*), the first row lists the values of $|\alpha_O-\alpha_{M'}|$ for each $h$ from 0 to 6; the second row lists the values of $\alpha_{M'}$ from Table 3, and the third and fourth rows list the values of $\alpha_O'$ and $\alpha_O''$ which are the two values of $\alpha_O$ compatible with the values of $|F_{O+M'}|^2$, $|F_O|^2$ and $F_{M'}$ used. Table 4 (*b*) presents the corresponding results obtained from $X'$; the last two rows are called $\alpha_O'''$ and $\alpha_O^{iv}$. A comparison at each $h$, of the values of $\alpha_O'$ and $\alpha_O''$ with those of $\alpha_O'''$ and $\alpha_O^{iv}$ reveals no consistent correspondence. It is possible, however, that these values of $\alpha_O$ are referred to different origins. (In fact, this is known to be true, from the way the data were obtained in this case.) If this is so, the differences between the $\alpha_O$'s obtained from the $M'$ structure and those obtained from the $X'$ structure should be of the form $h\beta$ (where $\beta = 2\pi x_0$ expressed in degrees and $x_0$ is the shift in origin between structures $M'$ and $X'$). Table 4 (*c*) presents all these differences, the four rows being, respectively: $\alpha_O'-\alpha_O'''$, $\alpha_O'-\alpha_O^{iv}$, $\alpha_O''-\alpha_O'''$ and $\alpha_O''-\alpha_O^{iv}$. It is seen that it is impossible to find a value of $\beta$ which will make a value of the difference in each column equal to $h\beta$. It is still possible, however, that not only are $M'$ and $X'$ referred to different origins but also correspond to different enantiomorphs. If this is so (and we know it is), all the values of $\alpha_O$ from either the $M'$ or the $X'$ data should be changed in sign, before taking the differences of Table 4 (*c*). In other words, instead of differences, sums should be taken; these sums appear in Table 4 (*d*), the four rows corresponding, respectively, to $\alpha_O'+\alpha_O'''$, $\alpha_O'+\alpha_O^{iv}$, $\alpha_O''+\alpha_O'''$ and $\alpha_O''+\alpha_O^{iv}$. It is seen at once that choosing $\beta = 60°$ expresses all the values in the third row in the form $h\beta$. (These numbers need not all have been in the third row, but might have varied from one row to another; that they are in one row is fortuitous.) This observation proves that structures $M'$ and $X'$ correspond to enantiomorphs of structure $O$, and that the origins were taken 60/360 apart, i.e. $x_0 = 2/12$.

In order to refer structures $M'$ and $X'$ to the same origin and have them correspond to the same enantiomorph of $O$, one of them must be changed to its mirror image and the origin of one of them moved by two-twelfths of the period. It is assumed that this treatment is to be given to $X'$. $X'$ has atoms at $x = 0$, 3/12, 10/12; changing these to their negatives gives $X''$, the enantiomorph of $X'$, with atoms at $x = 0$, 2/12, 9/12, $X''$ is still not referred to the same origin as $M'$; to accomplish this, each atomic coordinate in $X''$ must be increased by 2/12 to give structure $X'''$, with atoms at $x = 2/12$, 4/12, 11/12. The values of $F_{X'''}$, $\alpha_{X'''}$ and the corresponding two values of $\alpha_O$ (called $\alpha_O^{v}$ and $\alpha_O^{vi}$) are set forth in Table 4 (*e*).

It is seen at once that there is a perfect correspondence at each value of $h$ between $\alpha_O''$, obtained (Table 4 (*a*)) from $|F_O|$, $|F_{O+M}|$ and $F_{M'}$, and $\alpha_O^{vi}$, (Table 4 (*e*)) obtained from $|F_O|$, $|F_{O+X}|$ and $F_{X'''}$. Thus, structures $M'$ and $X'''$ are referred to the same origin, and correspond to the same enantiomorph of $O$. The values of $\alpha_O''$ (or, what is the same thing, $\alpha_O^{vi}$) are a self-consistent set of phase angles for the structure factors, $F_O$, of structure $O$. If angles $\alpha_O''$, from Table 4 (*a*), are compared with the original phase angles $\alpha_O$, from Table 2, it is found that $\alpha_O''(h) = -\alpha_O(h)+240^\circ h$, which means that structure $O$, as found 'correctly' from the data, is the enantiomorph of the true structure and is referred to an origin 8/12 of the period from that in the original description. This, however, is all that can be expected from a structure determination using X-ray diffraction data.

## 6. Generalization to three dimensions

The procedure just described is easily generalized to three dimensions. In general, structures $M'$ and $X'$, as found from the intensities of the X-rays diffracted by crystals with the structures $O$, $O+M$ and $O+X$, will be referred to different origins and are as likely as not to correspond to different enantiomorphs. The two different origins will be separated by a vector in the crystal which has components $x_0$, $y_0$ and $z_0$ and the differences or sums corresponding to the entries in Tables 4 (*c*) or 4 (*d*) will be of the form $2\pi(hx_0+ky_0+lz_0)$ when structures $M'$ and $X'$ refer to the same enantiomorph of $O$. In consequence of this, a sequence of reflections such as $(0kl)$, $(1kl)$, $(2kl)$,..., etc. can be used to find $x_0$ alone, since, for these, the entries will be of the form $h\beta+\Delta$, where $\beta$ is $2\pi x_0$ and $\Delta$ is $2\pi(ky_0+lz_0)$, both expressed in degrees. It is interesting —and advantageous—that this process can be carried through for each combination of $k$ and $l$, thus providing a multitude of checks on the value of $x_0$. Simultaneously, it will be discovered whether or not structures $M'$ and $X'$ correspond to enantiomorphs of $O$, and, if they do, one of them can be changed into its mirror image. In a similar way, the sequences $(h0l)$, $(h1l)$, $(h2l)$, ..., etc., and $(hk0)$, $(hk1)$, $(hk2)$, ..., etc., can be used to find $y_0$ and $z_0$, respectively. Of course, the phases of the various $F_O$'s are found simultaneously. Once $M'$ and $X'$ are properly located and referred to the same enantiomorph of $O$, all the phases of the $F$'s can be found. With this knowledge, it is then possible to compute directly the Fourier series for the electron density of structure $O$.

## 7. A more general treatment

The method for phase determination described in the preceding paragraphs has been based on the isomorphous replacement of vacancies in structure $O$ by the atoms of structures $M$ or $X$. This type of situation is rare in nature: in the more usual case, one kind of atom replaces another. Thus, one would usually be faced with a set of crystals with structures such as this: $O+M+X$, $O+M'+X$, $O+M+X'$ and, perhaps, $O+M'+X'$, where structures $M$ and $M'$ are the same, except that they are composed of different atoms, and similarly for $X$ and $X'$. Consider the pair of structures $O+M+X$ and $O+M+X'$. If we suppose, as is usually true in practice, that all the atoms in $X$ are of the same kind (for instance, chlorine atoms) and that the same holds for $X'$, but with a different kind of atoms (for instance, bromine atoms), then we have that

$$F_{X'} = \{f_{X'}/f_X\}F_X \tag{8}$$

and

$$F_{X'}-F_X = \{(f_{X'}-f_X)/f_X\}F_X \tag{9}$$

for each $(hkl)$. Thus we can write

$$F_{O+M+X'} = F_{O+M+X}+F_{X'-X}\,. \tag{10}$$

In most cases, the values of $F_{X'-X}$ can be found directly by comparing the Patterson functions of structures $O+M+X$ and $O+M+X'$, and, from these, working out the structure $X'-X$. This structure can be considered as substituted for a set of vacancies in structure $O+M+X$. Now, by using the experimentally determined values of $|F_{O+M+X}|^2$, $|F_{O+M+X'}|^2$ and $F_{X'-X}$, two solutions can be found for $F_{O+M+X}$. (This is done in the same way that two solutions are found for $F_O$ from the measured values of $|F_O|^2$, $|F_{O+X}|^2$ and $F_X$, as described previously.)

By using a parallel procedure, starting with the data obtained from structures $O+M+X$ and $O+M'+X$, two independent solutions can be found for $F_{O+M+X}$, from $|F_{O+M'+X}|^2$, $|F_{O+M+X}|^2$ and $F_{M'-M}$. If a common solution occurs in each pair, $F_{M'-M}$ and $F_{X'-X}$ are referred to the same origin and correspond to the same enantiomorph, if not, then a 'correct' origin and enantiomorph can be found by the methods outlined in previous paragraphs, and the values of $F_{M'-M}$, $F_{X'-X}$ and $F_{O+M+X}$ can all be referred to this origin and enantiomorph. Now, using

$$F_X = \{f_X/f_{X'-X}\}F_{X'-X} \quad \text{and} \quad F_M = \{f_M/f_{M'-M}\}F_{M'-M}\,, \tag{11}$$

we can find $F_O$ from the relation

$$F_O = F_{O+M+X}-F_M-F_X\,, \tag{12}$$

and, from this, structure $O$ can be found by summing a Fourier series.

## 8. The effect of variations from exact isomorphism

It has been assumed in the foregoing discussion that the lattice constants and atomic parameters of the crystals in an isomorphous series are all exactly alike; this state of affairs is not likely to be found in nature, although it is sometimes closely approximated. The question then arises: how far can two crystals differ from isomorphism without seriously vitiating the method of determining phases just described?

In order to make this question definite, let us assume that structure $O$ is a complicated organic molecule which maintains its dimensions and orientation with respect to the crystal axes, and that the effect of adding structures $M$ or $X$ is only to increase the lengths of the crystal axes. For convenience, we choose the origin at the centroid of scattering of structure $O$ and assume that the greatest possible value of $|x|$, $|y|$ or $|z|$ for an atom of $O$ is 0·5. Let us suppose that there are two equal atoms in $O$, with $x$ coordinates $+0{\cdot}5$ and $-0{\cdot}5$, respectively, when the lattice constants $a_0$, $b_0$ and $c_0$ of the crystal are at a minimum. As the lattice constants increase, by fractional amounts $\alpha$, $\beta$ and $\gamma$, respectively, these $x$ coordinates decrease by the fractional amount $\alpha$ (to a sufficient approximation). The contribution of these two atoms to the structure factors of the $h00$ reflections of the crystal is given by

$$2f \cos 2\pi h . \tfrac{1}{2}(1-\alpha) = 2f(-1)^h \cos \pi h\alpha .$$

This contribution never changes by more than 15% of its maximum range as $h\alpha$ changes by 0·1. All other pairs of atoms in structure $O$ contribute smaller changes to these structure factors. It seems reasonable to say that crystals will behave as isomorphous for purposes of phase determination if the change from one member of the series to another in the contribution of any atom to $|F_O|$ is less than 15% of its maximum contribution; consequently, $h\alpha$ (also $k\beta$ and $l\gamma$) should be held below 0·1. For instance, if the largest value of $h$ under consideration is 25, then $\alpha$ must be less than 0·004 (0·4%), etc.

There are other kinds of deviations from isomorphism, besides those due to changes in axial lengths; for instance, structure $O$ may be rotated or distorted by the addition of structures $M$ and $X$, without changing appreciably the lattice parameters of the crystal. Such deviations from isomorphism are almost impossible to detect *a priori*, but their importance can be minimized by the use of chemical sense. In any event, the effects of such deviations will be too small to spoil this phase-determination method if the change in $hx+ky+lz$ for any atom in $O$ and for any index triple $hkl$ is less than 0·05 between different crystals in the series. This criterion reduces to that of the last paragraph under the special assumptions stated there.

## 9. Absolute intensities

The whole of the argument presented here depends on having available the correct absolute intensities of X-ray reflection for all members of the isomorphous series of crystals under study. It is fairly easy to put all members on the same *relative* basis by dividing the measured intensities by the volumes of the crystals, since the unit cell volume does not change* throughout an isomorphous series. The factor converting to the *absolute* scale can then be determined by comparing the calculated and observed values of $F_M$, $F_X$, $F_{M'-M}$ or $F_{X'-X}$, after the corresponding structures have been found. Wilson's method (Wilson, 1942) for placing on an absolute scale the measured intensities of the X-rays diffracted by a crystal frequently gives results in error by as much as 50%; it is, consequently, more useful as an approximate check on the absolute scale, than as a method of determining that scale exactly. On the other hand, Wilson's method should work very well for placing the various members of an isomorphous series on the same *relative* scale, since the general structure of the crystals is here always the same, and the errors in this method are due to peculiarities of the structure.

## 10. Application to protein crystals

It has been found recently (King, Magdoff, Adelman & Harker, to be published) that crystals of several proteins react with solutions containing large organic dye molecules in such a way that these attach themselves to the protein molecules in definite positions, but without distorting appreciably the unit cells of the protein crystals. These dye molecules can be made to contain various arrangements of heavy atoms and so can, by themselves, furnish both structures $M$ and $X$. This is a particularly convenient way of forming these structures, since it can be known *a priori*, on stereo-chemical grounds, how $M$ and $X$ are related to one another in space and also, if they are asymmetrical, how corresponding enantiomorphs of each should be chosen.

It is probable that other ways can be found of relating structures $M$ and $X$, on the basis of *a priori* knowledge, which would be applicable to the solution of the structures of other non-centrosymmetrical crystal structures.

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* If the lattice constants change by such amounts as are allowed by the considerations of the previous paragraph, the change in cell volume is still completely unimportant.

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# A New Aid to the Determination of the Point-Group Symmetry of Transparent Crystals

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The patterns formed by electrical breakdown paths provide a new way to investigate the point-group symmetry of transparent crystals because the paths lie along equivalent directions. A point-group is uniquely determined from the observation in a single-crystal slice of non-degenerate breakdown configurations. Such non-degenerate configurations are formed in most monoclinic and in all triclinic crystals, while most crystals of higher symmetry reveal degenerate configurations which are compatible with more than one point group. Degeneracy, however, is not an inherent property of the breakdown process and might be removed by suitable techniques. Since the paths are believed to be formed by electron avalanches, the symmetry shown by the breakdown pattern probably refers to the symmetry of the electrical fields within the crystal that influence the motion of electrons. The symmetry of the breakdown pattern is usually identical with the symmetry of the crystal determined by other methods. A new test for the lack of centrosymmetry in transparent crystals is described.

## Introduction

This paper introduces the study of the patterns formed by electrical breakdown paths as a new aid to the determination of the point-group symmetry of transparent crystals. Each crystal shows an overall breakdown pattern that conforms with one or more point-group symmetries. Individual breakdown patterns, consisting of paths that lie along equivalent directions of the crystal, are called here breakdown configurations. Each configuration conforms with the symmetry of the overall pattern, but may differ from other configurations in orientation and in number of paths. Since the paths are believed to be formed by electron avalanches, the symmetry revealed by the pattern is probably the symmetry of the electrical fields within the crystal that influence the motion of electrons. Although, for crystals of high symmetry, the method suffers from the same limitations that are encountered in morphological studies, in that 'special forms' (here called degenerate configurations*) tend to develop, it is sometimes possible to obtain less degenerate configurations, or non-degenerate configurations, by changing the conditions of breakdown, such as by changing the temperature of the crystal or by applying overvoltages. While the degeneracy is seldom completely removed, the partial information that is obtained is usually sufficient to show that certain point groups are incompatible with the pattern. The incompatible groups may have higher or lower symmetries than the true symmetry of the crystal for the breakdown process, depending upon the nature of the degeneracy. If one then gains knowledge by some other means of the existence of those symmetry elements which are not established by the degenerate configuration, or, if one obtains a non-degenerate breakdown configuration, a point-group symmetry is uniquely determined. Since the breakdown pattern lies in three dimensions, its full symmetry is revealed in a single-crystal section or slice. Thus euhedral crystals are not necessary for the symmetry determination.

The point-group symmetry is most simply determined by examining the breakdown configurations in

* A degenerate electrical breakdown path configuration is one that consists of paths that lie in planes or along axes that could be symmetry operators. When the paths are in such positions it is not possible to determine whether or not the planes or axes are symmetry operators for the breakdown process. Hence degenerate configurations are compatible with two or more point-group symmetries. When all configurations are degenerate the overall pattern is degenerate and the point-group symmetry of the crystal for the breakdown process is not established.