Isomorphous Replacement Method in Non-centrosymmetric Structures

By Gopinath Kartha

*Roswell Park Memorial Institute, Buffalo 3, N.Y., U.S.A.**

Even though the advantages of the isomorphous replacement method over the heavy atom method have been recognized for a long time, this method has not had as wide an application in solving non-centrosymmetric structures as the heavy atom method. Viewing the problem of structure analysis as that of 'unfolding' the vector map of the structure, it is seen that in principle the isomorphous series method should have exactly the same applicability as the heavy atom method in the case of non-centrosymmetric structures also. A Fourier synthesis based on data from two non-centrosymmetric isomorphous crystals is suggested and this synthesis is shown to be of considerable value in solving such structures.

Introduction

Some of the most successful analyses of complicated organic crystal structures have been achieved with the help of either the isomorphous replacement method or the heavy atom method. Even though at first sight these two methods seem very different with regard to their applicability in any particular case, more detailed study shows that the principles used in deducing the structure from the measured intensities of reflections are almost identical in the two cases. In theory, the heavy atom technique applied to a crystal with an infinitely heavy atom and with intensities measured with no errors will give the same results as the isomorphous series. Hence, the isomorphous series will have the same applicability, limitations and ambiguities as the corresponding idealized heavy atom method. The advantage of the isomorphous series method over the heavy atom method is in the accuracy with which the light atom positions can be determined for a given accuracy in the intensity measurements. Indeed, this fact has been recognized by crystallographers for a long time and applied in many successful structure analyses. However, in all these analyses, the isomorphous technique has been applied to the centrosymmetric cases; the heavy atom method being more widely used in non-centrosymmetric cases *even when isomorphous crystals were easily available*. Regarding the problem of structure analysis of isomorphous crystals as a problem in 'unfolding' the difference Patterson (D.P.) of the structure, it has been shown (Kartha & Ramachandran, 1955) that this can be done as easily in the acentric as in the centric case, and that it is possible to recover the original structure from the (D.P.) in 219 of the 230 space groups, if the replaceable atoms are in general positions. For the remaining 11 polar space groups, the structure duplicated by its inverse

is obtained if there is only one replaceable atom per asymmetric unit. This ambiguity can also be removed if the structure contains more than one replaceable atom per asymmetric unit. The same treatment applies to the Patterson of the heavy atom structure and the same limitations also apply. It is thus clear that, viewing the problem of structure analysis as an 'unfolding' problem, there is no difference in principle between the two methods as applied to either the centric or non-centric cases.

It was pointed out in the paper cited above (Kartha & Ramachandran, 1955) that the problem of non-centric structures viewed as an 'unfolding' problem using superposition and minimum function methods can be solved without ambiguity, whereas trying to solve the problem by determining the phases of the reflections gave ambiguous results. However, it was felt that two isomorphous non-centrosymmetric crystals contained enough information to 'arrive' at the structure by the phase method, provided the problem is suitably formulated. With this aim the author made a detailed study of the way in which the simplified Patterson, containing only vectors involving heavy or replaceable atoms can be 'unfolded' by a superposition method to enable a complementary Fourier series formulation using measured amplitudes and inferred phases, which also gives the same solution. As a result, a modified Fourier series is obtained which extends the isomorphous series method to non-centric cases, so that we can make use of the obvious advantages of this method over the more commonly used heavy atom method. As the success of the suggested Fourier is capable of a surprisingly simple intuitive explanation, such an argument leading to the suggested series is developed in the next sections.

Phase ambiguity in non-centric structures

If we consider the vector diagram (Fig. 1) describing the scattering from two isomorphous crystals C_1 and

---

* Work was begun at the Polytechnic Institute of Brooklyn, Brooklyn, N.Y., U.S.A.

C_2, it is seen that there are two possible solutions corresponding to two values for the phase angle φ of the scattering amplitude $|F|$ from that part of the structure common to the two isomorphs. These two values may be written

$$\varphi = \alpha \pm \theta \, , \qquad (1)$$

where θ is given by

$$\cos\theta = |F_1|^2 - |F_2|^2 - |R_1|^2 + |R_2|^2/2|F||R| \, . \qquad (2)$$

The quantities on the right hand side of (2) are indicated in Fig. 1. It is seen that the two possible values for the phase angle φ are symmetrical about α, the phase of the replaceable group.
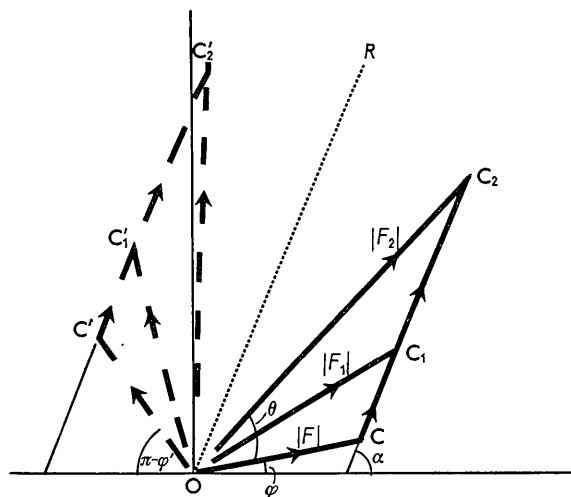

Fig. 1. Phase ambiguity in non-centric structures. The amplitudes $|F_1|$ and $|F_2|$ for the two crystals C_1 and C_2 are known. Magnitudes $|R_1| = CC_1$ and $|R_2| = CC_2$ of replaceable atoms and phase angle α are also known. Aim is to determine phase φ and magnitude $|F|$ of unknown part C. Two possible vector solutions are given by OC and OC'.

In centrosymmetric cases no ambiguity arises, since $\theta = 0$ or π and in either case $-\theta = +\theta$ so that both solutions for φ in equation (1) coincide. In non-centrosymmetric cases this ambiguity in φ could be resolved by having another isomorphous crystal with atoms substituted in a different place. Using this we could get another possible pair of values for φ which are symmetrical about another value of α. In such a case one of the values of φ, the true one, should be the same for the two substitutions; of course allowing for the experimental error, and thus the correct value of φ could be picked. This method, the so-called multiple isomorphous series method, of determining phases in non-centrosymmetric cases has been suggested earlier by Harker (1956) and used in the analysis of proteins by Kendrew et al. (1958). The difficulty in the use of this method in ordinary organic structures is the chemical problem of obtaining more than two isomorphous crystals with additional atoms in different positions. Though it may be quite easy

to get three isomorphous salts, say of HCl, HBr and HI for many substances, this is not sufficient to resolve the ambiguity, as the halogen atoms are likely to be in similar positions and thus would lead to the same two values of φ as from the first substitution. Hence, the requirement that the crystals must have substituents in different positions and still be isomorphous is likely to be the highest hurdle in the applicability of the multiple isomorphous series method to ordinary organic structures. However, in the case of proteins, where the conditions and definition of isomorphism are much less stringent, it has been possible to use the method by attaching groups of heavy atoms at different specific sites on the molecule without much change in the general arrangement of the rest of the structure, at least to the resolution under study.

Double phased Fourier

In the series proposed in this paper for use with isomorphous non-centric crystals, the procedure is as follows. Using data from the two isomorphous crystals the phase angle φ can be calculated according to equations (2) and (1) except for an ambiguity in the sign of θ. In the absence of any additional data to resolve this ambiguity, it is proposed that a series computed using both the possible phases, one of which is known to be correct and the other wrong, will give useful results. It is reasonable to expect that the terms with the correct phase will continuously build up peaks at the true atomic positions, whereas the wrong phases do not in general build up systematically, but give rise to only dispersed spurious peaks of lesser height on a general background. The detailed analysis of the process of 'unfolding' the Patterson and reducing the background of spurious peaks to a minimum did indeed lead to this series and showed that this in fact is the case. It also indicated the positions and weights of the spurious peaks.

The series using both phases is given by

$$\begin{aligned}
\varrho(x, y, z) \\
= \Sigma\Sigma\Sigma |F| \, & [\exp[+i(\alpha+\theta)] + \exp[+i(\alpha-\theta)]] \\
& \times \exp -2\pi i(hx+ky+lz) \\
= \Sigma\Sigma\Sigma (2|F| & \cos\theta) \exp[i\alpha] \exp -2\pi i(hx+ky+lz) \\
= \Sigma\Sigma\Sigma F' & \exp -2\pi i(hx+ky+lz-\alpha) \, . \qquad (3)
\end{aligned}$$

Equation (3) shows that the double phased Fourier can be written as an ordinary series with F' as amplitude and α, the phase of the replaceable group, as the phase angle. Here F' is given by expression (4)

$$F' = 2|F| \cos\theta = |F_1|^2 - |F_2|^2 - |R_1|^2 + |R_2|^2/|R| \qquad (4)$$

and can be evaluated from the unknown quantities on the right hand side. Equation (3) can also be regarded as a Fourier series with coefficient $|F|$ weighted with the weighting factor $\cos\theta$ and having the phase angle α of the replaceable group. It is interesting to note

that the series given in (4) is the same as the one denoted 'isomorphous β synthesis' by Ramachandran & Raman (1959) and arrived at by different considerations.

It has been suggested above that by performing a Fourier synthesis using both the correct and incorrect phases, the correct phases tend to build up peaks at the actual atomic positions, while the incorrect ones cause only minor peaks in the background. However, this fact is by no means obvious and there are cases where the incorrect phases also consistently add up to an equally high density at positions which do not correspond to any actual atomic position. For example, in the case where the replaceable set of atoms has a centre of inversion while the structure itself does not, we have the phase α of the contribution of the replaceable atoms always 0 or π. In such a case, of the two phases $\varphi = \alpha \pm \theta$, the incorrect phase consistently cancels out the imaginary 'B' component of all reflections, thus superposing a centrosymmetrically related ghost structure of equal weight over the true set of peaks. This, in fact, was the case with the analysis of the strychnine molecule (Bokhoven, Schoone & Bijvoet, 1951) where the duplication occurred with each atom represented by a pair of equally dense peaks, one of which had to be discarded and the choice had to be made by using the results from a centrosymmetric projection and a knowledge of interatomic distances. This shows that a more detailed study of the effect of the introduction of Fourier coefficients with incorrect phases is necessary, before we can comment on the applicability of the method.

Peaks due to incorrect phases in Fourier

If we assume that the structure C is obtained by the Fourier transformation of the structure amplitudes $|F|$ with correct phases $\alpha + \theta$, we shall try to find the implication of the series involving $|F|$ and the incorrect phase $\alpha - \theta$, i.e.,

$$\text{F.T. of } |F| \exp[i(\alpha + \theta)] = \mathbf{C} . \qquad (5)$$

Hence

$$\begin{aligned} &\text{F.T. of } |F| \exp[i(\alpha - \theta)] \\ &= \text{F.T. of } \{\exp[2i\alpha]\}\{|F| \exp[-i(\alpha + \theta)]\} . \end{aligned} \qquad (6)$$

By Fourier transform theory the F.T. of the right hand side of equation 6 is the convolution of the F.T.'s of the terms in the curly brackets. Obviously, the F.T. of $|F|$ with phase angles the negative of those of C is the inverse of structure C, which we might denote by C*. In the language of image theory this means the incorrect phases give rise to ghost images of the inverse structure C* repeated at points corresponding to the Fourier transform of $\exp[2i\alpha]$.

Let us try to find peaks corresponding to the $\exp[2i\alpha]$ series. We know that the series $|R_1| \exp[i\alpha]$ gives peaks of weight R_i at the positions r_i correspond-

ing to the replaceable atoms of the first crystal. A Fourier series with $|R_1|^2 \exp[2i\alpha]$ will give peaks of weight $R_i R_j$ at positions $r_i + r_j$. This set of peaks can be divided into two groups. The first corresponds to the diagonal elements of the set $R_i R_j$ with weight $|R_i|^2$ at position $2r_i$ and these are n in number where n is the number of replaceable atoms. The second group, $n(n-1)/2$ in number corresponds to the off-diagonal elements, which are all double peaks and have weights $2R_i R_j$ and positions $r_i + r_j$.

As a first approximation, the positive peaks of the $\exp[2i\alpha]$ series will have the same configuration and relative weights as the $|R|^2 \exp[2i\alpha]$ series, but with weights reduced by a factor $1/\Sigma R_i^2$. Thus we might write out the peaks of the $\exp[2i\alpha]$ series as

(1) n single peaks of weight $R_1^2/\Sigma R_i^2$ at $2r_i$,
(2) $n(n-1)/2$ double peaks of weight $2R_i R_j/\Sigma R_i^2$ at $r_j + r_j$. $\qquad (7)$

Thus, the resultant peaks from the double phased synthesis in equation (3) for the case where all R atoms are similar are:

(a) One image of structure C with weight unity at origin,
(b) n images of inverse structure C* with weight $1/n$ at positions $2r_i$,
(c) $n(n-1)/2$ images of structure C* with weight $2/n$ at positions $r_i + r_j$. $\qquad (8)$

Of the three sets of images in (8) resulting from the double phased synthesis (8)(a), is due to use of the correct phases while (8)(b) and (8)(c) are due to incorrect phases. It is seen that the required image of C has a higher weight than all the ghost images of the inverse of C in all cases where there are more than two replaceable atoms in the unit cell. For special configurations of the replaceable group R, either due to accident or symmetry, the peaks in (8)(c) may coincide, causing superposition of the inverse structure C*. This happens if, for any pair of R atoms, there are double or multiple peaks in the Patterson of this set; so that $(r_i - r_j) = (r_l - r_n)$. This causes another pair of interactions in the set (8)(c) (namely $r_i + r_l = r_j + r_n$) to coincide and the corresponding weight of the off-diagonal C* image becomes $2(R_i R_l + R_j R_n)/\Sigma R_i^2$ which reduces to $4/n$ for the case of similar R atoms. Of course, this number will always be less than $n/n = 1$ and hence will never be greater than the weight of the image of C given by (8)(a). Thus, multiple peaks $ij = ml$ in the Patterson of the R group give corresponding multiple peaks $im = jl$ of weight $4/n$ in the set (8)(c). The only time any peak ij in set (8)(c) is as large as unity is when the whole set of atoms in the group R can be divided into two sets of $n/2$ each, such that

$$R_1 + R_{(n/2+1)} = R_2 + R_{(n/2+2)} = \ldots = R_{(n/2)} + R_n = 2q \text{ (say)}$$

in which case we can write these as

$$R_1 - q = q - R_{(n/2+1)}; \quad R_2 - q$$
$$= q - R_{(n/2+2)}, \ \ldots, R_{(n/2-1)} - q = q - R_n .$$

With such an arrangement, the peak in (8)(c) corresponding to the interaction between the two groups will coincide at the point $2q$ giving a multiple peak of weight n/n i.e.—the same as the weight of the structure C. Hence, in this special case, the Fourier synthesis with both the phases gives the inverse structure C* as seen from the point $2q$ and the true structure C imaged at the origin, both occurring with the same weight. Of course, this is the arrangement that one obtains for a centrosymmetric grouping of R atoms around q and, as has been shown by Kartha & Ramachandran (1955), under these conditions the structure is always accompanied by its inverse. The same can also be predicted by considering equation (6). In the case of the centric arrangement of the R groups centered about the origin (a case with $q=0$) the phase angle $\alpha = 0$ or π and in either case $\exp[2i\alpha] = $ unity and the corresponding Fourier transform is a function having non-zero values only at the origin and the weight of the corresponding inverse image is unity. Hence the incorrect phases give the inverse structure C* which has the same weight as the image C due to the correct phases.

Results from the vector map viewpoint

The principles involved in the above method can perhaps be somewhat more clearly followed by regarding the corresponding steps from the vector map viewpoint, and in the present section this is done diagrammatically for a simple structure. (Point atoms are used for clarity.) Fig. 2 shows the fundamental set of points (Buerger, 1950) divided into two types, type C which remains the same in both of the isomorphs, and the type R corresponding to the replaceable group of atoms. If we had both the phases and
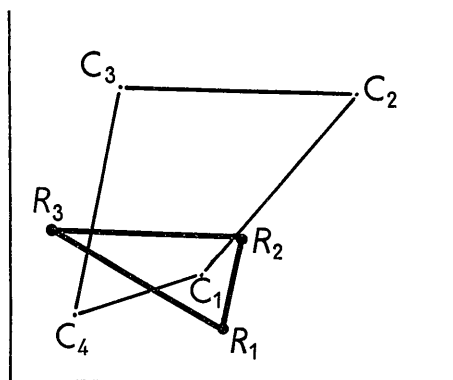

Fig. 2. Fundamental structure. Result of Fourier with amplitude $|F_1|$ if all phases φ were known. The known positions of replaceable group R represented by thick lined triangle. Unknown part C represented by vertices of thin lined polygon. Our aim is to recover this polygon C from measured quantities.

A C 14 — 44

amplitudes of all the reflections of one crystal, say C_1, then a Fourier series involving these will give us peaks corresponding to the structure shown in Fig. 2. However, as the phases are unavailable, our aim is to get the polygon represented by the unknown part of the structure from a knowledge of the amplitudes $|F_1|$ and $|F_2|$ for the two isomorphs and also of the positions and scattering contributions of the replaceable groups of atoms R_1 and R_2.
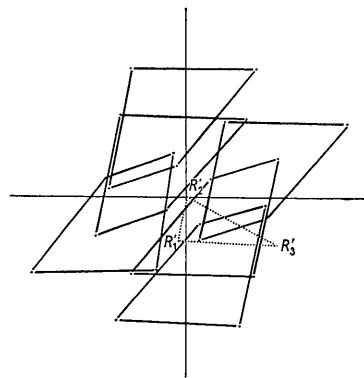

Fig. 3. Simplified Patterson map where all vectors which do not involve replaceable atoms are removed. This is the result of a series with $(|F_1|^2 - |F_2|^2 - |R_1|^2 + |R_2|^2)$.

The 'simplified Patterson' can be derived from a synthesis with coefficients $(|F_1|^2 - |F_2|^2 - |R_1|^2 + |R_2|^2)$ and the peaks corresponding to this are shown in Fig. 3. This synthesis contains only the images of the unknown part C of the structure as seen from all points of R and also their inverses.

The result of unfolding this simplified function by the weighted sum function method (Kartha & Ramachandran, 1955) is given in Fig. 4 and, from the phase method point of view, this map is the result of a synthesis with $(|F_1|^2 - |F_2|^2 - |R_1|^2 + |R_2|^2)|R|$ as amplitude and taking the phase α the same as that of the replaceable group of atoms (Ramachandran & Raman, 1959). It will be seen that in this case where the number of R group atoms $n=3$, the wanted image C occurs with maximum weight 3 and is accompanied by the following ghosts; 6 of C with weight 1, 3 of the inverse of C with weight 2 and another 3 of the inverse of C with weight 1. In the general case of n replaceable atoms the wanted structure C has n fold weight and is accompanied by $n(n-1)$ images of C with weight 1, $n(n-1)/2$ of C* with weight 2 and another n images of C* with weight 1. These images of C corresponding to Fig. 4 could be more easily visualized by considering Fig. 5 where each image polygon is denoted by a single representative point of appropriate weight. It will be seen that Fig. 5 is a map with $|R|^2 + R^2$ as coefficients. The first term gives rise to the Patterson peaks $r_i - r_j$ of the replaceable group. This, as is well known, has a large peak of weight n at the origin and $n(n-1)$ non-origin peaks which are, in general, single. The second set of

peaks due to $\mathbf{R}^2 = |\mathbf{R}|^2 \exp[2i\alpha]$ corresponds to peaks at points $\mathbf{r}_i + \mathbf{r}_j$ which are in general of double weight, except for the case $i = j$ when the corresponding peaks at $2\mathbf{r}_i$ are single. The map in Fig. 4 may be regarded
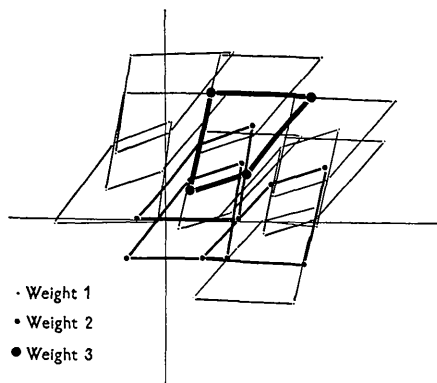

· Weight 1
• Weight 2
● Weight 3

Fig. 4. Result of superposition of Fig. 3 at inverse points of R group denoted by dotted triangle in that figure. This weighted sum function will be given by series $(|F_1|^2 - |F_2|^2 - |R_1|^2 + |R_2|^2)|R|$. Weight of various images are indicated.

· Weight 1
• Weight 2
● Weight 3
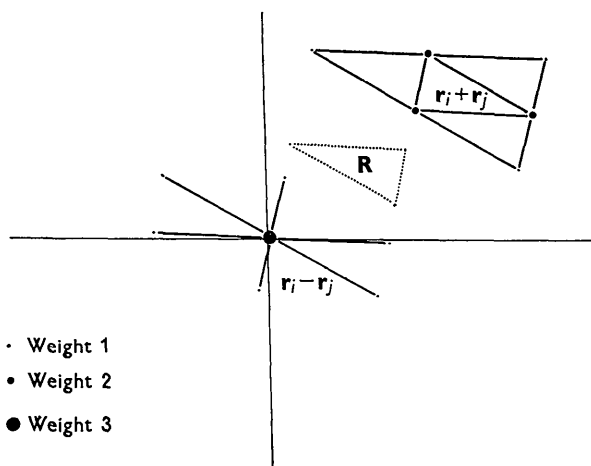
Fig. 5. Weight and position of images in Fig. 4. R group shown by dotted triangle. Points of repetition could be divided into: a; set $\mathbf{r}_i - \mathbf{r}_j$ and b; set $\mathbf{r}_i + \mathbf{r}_j$.

as the repetition of the structure $\mathbf{C}$ at the points of the Patterson peaks $\mathbf{r}_i - \mathbf{r}_j$ of the replaceable group and images of the inverse of $\mathbf{C}$ at the points $\mathbf{r}_i + \mathbf{r}_j$ given by the second set; the images occurring with the appropriate weights.

As a first approximation, the set of positive peaks of the double phased synthesis given in equation (3) is equivalent to that obtained by removing $n(n-1)$ images due to the non-origin peaks of the Patterson $(\mathbf{r}_i - \mathbf{r}_j)$ in Fig. 5. The resulting synthesis thus removes all unwanted images of $\mathbf{C}$ from Fig. 4 and the result is shown in Fig. 6. Thus, the double phased synthesis contains a single image of the wanted structure $\mathbf{C}$ with weight n. The accompanying ghosts are the
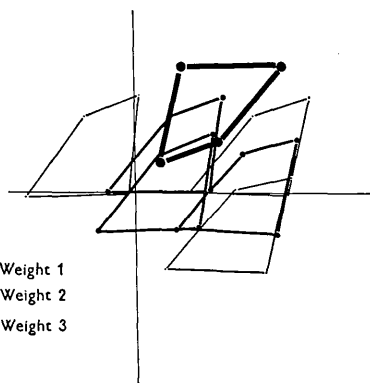

· Weight 1
• Weight 2
● Weight 3

Fig. 6. Result of removing non-origin peaks of set $\mathbf{r}_i - \mathbf{r}_j$ in Fig. 5. This map is obtained by using both phases indicated by isomorphous replacement, i.e., it has amplitude $|F|\cos\varphi$ and phase α.

images of the inverse of $\mathbf{C}$ repeated at the points $\mathbf{r}_i + \mathbf{r}_j$ shown in Fig. 5. With the data available for two non-centric isomorphous crystals, this seems to be the maximum simplification possible in removing ghost peaks and accentuating the wanted part.

Application of the method

The method has been tested on the [001] projection of a hypothetical crystal with a triclinic unit cell with $a = 12$, $b = 15$ Å, $\gamma = 82°$ and space group P_1. The two isomorphous crystal $\mathbf{C}_1$ and $\mathbf{C}_2$ both contain twenty carbon atoms at identical positions. The first crystal also contains in addition three atoms in known positions, their scattering power being twice that of a nitrogen atom. For this structure, shown in Fig. 7, the quantities $|F_1|$, $|F_2|$, $|R|$ and α were calculated over the whole region within the copper $K\alpha$ reflecting
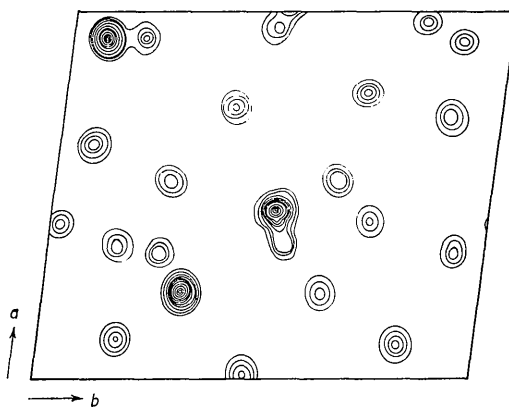

Fig. 7. Structure assumed for testing the method. Cell constants $a = 12$, $b = 15$ Å and $\gamma = 82°$. Contains twenty carbon atoms as unknown part and three double weight nitrogen atoms as replaceable group. All atoms assumed to have isotropic temperature factor $B = 2$.

circle.* A uniform temperature factor parameter $B=2$ was assumed for all the atoms.
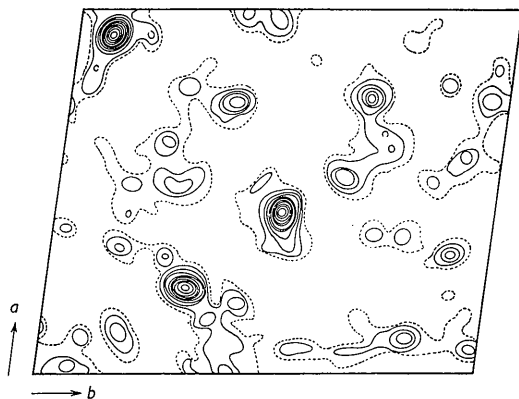

Fig. 8. Fourier with heavy atom phases. Except for heavy atom peaks most of the remaining peaks are in incorrect positions.

First an attempt was made to solve the structure **C** as a heavy atom problem by giving to the amplitudes $|F_1|$ the phases of the scattering from the three double nitrogen atoms. The resulting map is shown in Fig. 8. Some peaks, about eight, did show up at the required positions, but a larger number of equally high spurious peaks also appeared, so that, if we assigned the highest twenty peaks as true atomic positions, most of them would be wrong and refinement on this basis is not possible. This suggests that the heavy atom method using moderately heavy atoms (say, one chlorine atom against twenty carbon atoms) is not very efficient as a starting point for refinement in the case of non-centrosymmetric structures containing a fair number of light atoms.
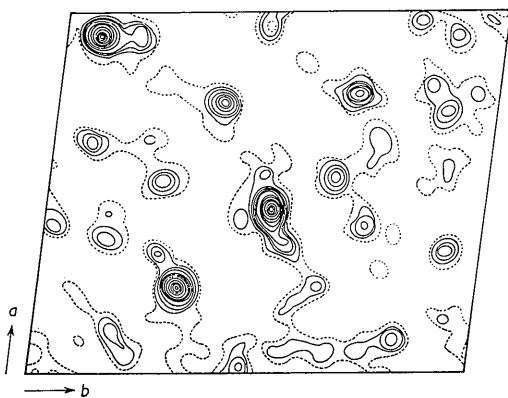

Fig. 9. Fourier with amplitude $|F_1|$ and both possible phases φ_1 for the vector OC_1. All twenty-three true atomic positions correspond to positive peaks and no spurious peak is as high as a real peak.

* The author's thanks are due to Dr Alexander Tulinsky for assistance in performing the computations in this section on the IBM 650 at the Watson Scientific Computing Laboratory in New York.
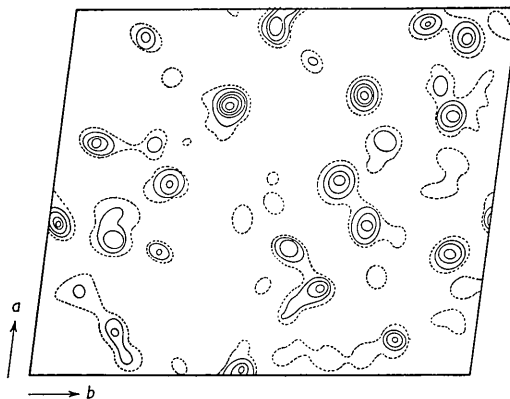

Fig. 10. Fourier with modified coefficients to remove the heavy atoms also. The twenty highest peaks occur exactly at the same place as the assumed light atom positions.

The Fourier synthesis for structure C_1 using both the possible phases was next made and is shown in Fig. 9. A similar series with slightly modified coefficients for the second crystal C_2 without the heavy atom is given in Fig. 10. Comparison with Fig. 7 shows that all the twenty highest peaks come exactly at the required positions and all spurious peaks are indeed smaller than the smallest true peak. Further, as shown before $(8)(a)$, (b), (c) this series is expected to give the true structure with weight three and the inverse structure repeated at three points with double weight and at another three points with single weight. The positions and weights of the peaks expected from theory are shown in Fig. 11 and comparison with Fig. 10 shows that the ghost peaks due to the use of incorrect phases all occur at the positions and with the intensities expected from the theory; quite a satisfying result. It may be recalled that the number of replaceable atoms in the unit cell chosen here, namely, 3, is the absolute minimum necessary in this method to form an acentric grouping of **R** atoms and that in practical cases the number will usually be higher than this, giving a correspondingly higher contrast between
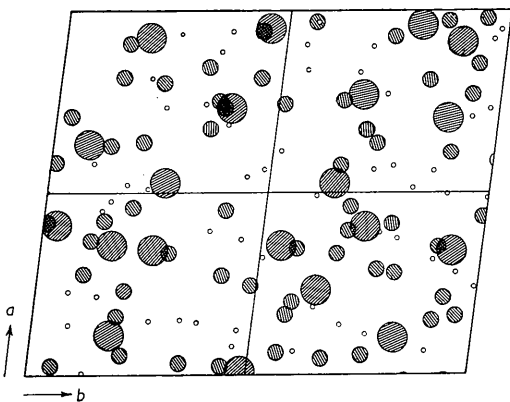

Fig. 11. Expected positions and weights for both real and spurious peaks in the double phased Fourier. Comparison with Fig. 10 shows agreement for these positions.

the true and ghost peaks. From the present case it seems that even the minimum of three replaceable atoms per cell makes the wanted peaks stand out against the background of a large number of double weight ghost peaks, 2 being the usual weight of these peaks even when the number of replaceable atoms is more than three. However, the number of ghost peaks per unit cell increases approximately as the square of the number of replaceable atoms per unit cell, and hence the probability of accidental coincidences of centers of ghost peaks and also the corresponding strengthening of the background of ghost rapidly increases. In any case, it may be expected that for average atoms which are not too diffuse and with three-dimensional data this effect may not be an important factor in most practical cases of isomorphous pairs where the number of replaceable atoms is only a small fraction of the total number of atoms in the cell.

Of course, by choosing a hypothetical case like the one here and calculating the $|F|$ values we have taken an ideal case of perfect isomorphism and no experimental errors in intensity measurements, but there seems to be all reason to be optimistic that the method will work in practical cases where random errors are allowed in intensity measurements. The method has in fact been applied (Tulinsky, 1960) with remarkable success to a very large molecule of unknown chemical formula and containing over fifty atoms, using data from the hydrochloride and the hydrobromide salts, both of which crystallized as non-centrosymmetric isomorphous structures. It was reported that the first double phased synthesis showed up a good part (35 atoms out of approximately 50) of the structure which could be chemically identified and these were used as the starting point for subsequent refinements. It is extremely doubtful whether the bromine atom in the bromide of such a large molecule would have been heavy enough to give a suitable trial structure by the application of the usual heavy atom method.

Conclusion

Using two isomorphous non-centrosymmetric crystals we get the amplitude $|F|$ and the phase $\varphi = \alpha \pm \theta$, the sign of θ alone being uncertain. In this respect the situation is similar to the sign problem of a single centrosymmetric crystal whose amplitude $|F|$ is known, but its sign alone is uncertain. In centrosymmetric structures this problem of the sign of $|F|$ can be solved either by using the well known isomorphous series method or by the trial structure method where a trial structure is proposed for the crystal, the phase angle of $|F|$ calculated on this basis and these phases are used

with the measured $|F|$'s in the refinement of the structure. In an essentially parallel way two methods may be used for determining the sign of θ in the phase angle expression for the case of two isomorphous non-centrosymmetric crystals. The first would be to use the multiple isomorphous series method (Harker, 1956) by producing, if possible, a third isomorph with the replaceable atoms in another set of positions. However, in ordinary cases this may be very difficult to achieve and hence we have to fall back on the second method, namely, the trial structure method, to resolve the sign ambiguity for θ. Fortunately this is a possible approach, since we already have data from two isomorphs and could produce a trial structure by the method of the double phased Fourier suggested here. Refinement could proceed with this trial structure, just as in the case of centrosymmetric structures, by using the trial structure phase angle φ', not directly in the next synthesis, but only for deciding the sign of θ to be used in the phase angle expression $\varphi = \alpha \pm \theta$. This type of refinement could be faster than the refinement of φ in the conventional heavy atom and trial structure methods in non-centric structures, the rate of convergence being similar to that of a centrosymmetric case in the earlier stages. However, during the final stages of refinement, due to lack of isomorphism at high resolutions, it may be advisable to apply the usual method of coordinate refinement using data from one crystal alone.

References

BOKHAVEN, C., SCHOONE, J. C. & BIJVOET, J. M. (1951). *Acta Cryst.* **4**, 275.

BUERGER, M. J. (1950). *Acta Cryst.* **3**, 87.

HARKER, DAVID (1956). *Acta Cryst.* **9**, 1.

KARTHA, G. & RAMACHANDRAN, G. N. (1955). *Acta Cryst.* **8**, 195.

KENDREW, J. C., BODO, G., DINTZIS, H. M., PARRISH, R. G., WYCKOFF, H. & PHILIPS, D. C. (1958). *Nature, Lond.* **181**, 662.

RAMACHANDRAN, G. N. & RAMAN, S. (1959). *Acta Cryst.* **12**, 957.

RAMAN, S. (1959). *Acta Cryst.* **12**, 966.

TULINSKY, A. (1960). (Private Communication.)