THE USE OF ANOMALOUS SCATTERING OF NEUTRONS IN THE SOLUTION OF CRYSTAL STRUCTURES CONTAINING LARGE MOLECULES*

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1. INTRODUCTION

THE effectiveness of anomalous dispersion methods for phase determination in X-ray crystallography has been established beyond question. The anomalous dispersion effects are much more pronounced in the case of neutron scattering than in X-ray scattering. This opens up immense possibilities of using anomalous neutron scattering for solving structures of very large molecules. The essential differences between X-ray and neutron scattering are discussed in this paper and some novel ways in which the anomalous neutron scattering could be exploited for the solution of the phase problem are proposed.

2. ANOMALOUS SCATTERING OF X-RAYS

When the wavelength of the incident X-ray beam is close to an absorption edge λ_k of an atom, the atomic scattering factor may be expressed as

$$f = f_0 + \triangle f' + i \triangle f'',$$

f_0 is the normal scattering factor for wavelengths far from the absorption edge, $\triangle f'$ is the real part and $\triangle f''$ the imaginary part of the dispersion corrections. The magnitudes of both these quantities are small compared to f_0. The variation of $\triangle f'$ and $\triangle f''$ with wavelength is illustrated in Fig. 1.

Coster, Knol and Prins[1] established that this complex scattering factor leads to the violation of Friedel's law in non-centrosymmetric structures. Bijvoet and his collaborators[2] showed how this effect [$I(hkl) \neq I(\bar{h}\bar{k}\bar{l})$] can be used to determine the absolute configuration of crystals. They also pointed out the possibility of using this inequality for determining the phases of the reflections. The analytical expression relating the phases and the observed
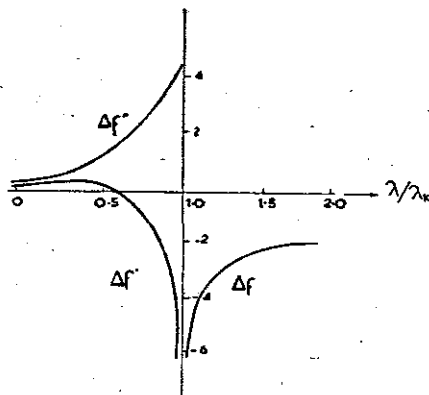
intensities was given by Ramachandran and Raman.[3]

FIG. 1. Variation of $\triangle f'$ and $\triangle f''$ with wavelength in X-rays near an absorption edge.

Mark and Szilard,[4] demonstrated that anomalous scattering of X-rays without phase change ($\triangle f'$ negative) is equivalent, in effect, to reducing the scattering power of the atom. Ramaseshan, Venkatesan and Mani[5] showed that if a pair of wavelengths on either side of the absorption edge of one of the species of atoms in the structure is used, the situation is equivalent to collecting data using a *single* wavelength with two *perfectly* isomorphous structures. They showed in a simple case that all the techniques of isomorphous replacement methods could validly be adopted. It was also suggested by them that when $\lambda_1 < \lambda_k < \lambda_2$, $[F(hkl)]_{\lambda_1}$. $[F(\bar{h}\bar{k}\bar{l})]_{\lambda_1}$ and $[F(hkl)]_{\lambda_2}$ could in principle be combined to determine the phase of the hkl reflection. For a review on anomalous X-ray scattering *see* Ramaseshan.[6]

3. ANOMALOUS NEUTRON SCATTERING†

That neutrons also are scattered anomalously by some nuclides was shown in a series of

careful experiments by Peterson and Smith.[7] They repeated the classical experiment of Coster, Knol and Prins with neutrons scattered by $Cd^{113}S$ and demonstrated the violation of Friedel's law. They showed that the anomalous scattering length could be written as $b = b_0 + b' + i\,b''$ and by comparing the X-ray and neutron scattering data they found that b'' leads $(b_0 + b')$ in phase by $\pi/2$, exactly as in the case of X-rays. They pointed out that Li^6, B^{10} and Cd^{113} which scatter thermal neutrons anomalously could be useful in determining the absolute configuration of crystals by neutron scattering. This was indeed borne out by Johnson, Gabe, Taylor and Rose[8] who determined the absolute configuration of the Li-salt of the enzymatically formed α-mono-deutero glycolate using neutron diffraction. Peterson and Smith also suggested that the phase problem in neutron scattering could be solved by the methods suggested by Bijvoet in X-ray scattering.**

The scattering length for neutrons in the resonance region is given by the Breit-Wigner[10] formula ‡

$$b = R + \frac{1}{2}\,\frac{g\,\omega\,\lambda_0\,\Gamma_n\,(E - E_0)}{(E - E_0)^2 + \Gamma^2/4}$$
$$+ i\,\frac{1}{4}\,\frac{g\,\omega\,\lambda_0\,\Gamma_n\,\Gamma}{(E - E_0)^2 + \Gamma^2/4}$$

where g is the spin weighting factor, $\lambda_0 = \lambda/2\pi$, Γ_n is the neutron width, Γ the total width, E the energy of measurement, E_0 the resonant energy and R the weighted mean of the nuclear radius corresponding to potential scattering. Brockhouse,[11] who measured the scattering cross-sections for neutrons of different energy, found that, for most nuclides, one-level Breit-Wigner formula fitted the experimental data very well. Figure 2 gives the curves for b' and b'' for Cd^{113} as calculated from the measurements of scattering and absorption cross-sections made by Brockhouse. The essential features of these curves have been verified by Peterson and Smith by accurate measurements of intensities of $I(hkl)$ and

$I(\bar{h}\bar{k}\bar{l})$ in $Cd^{113}S$ for different wavelengths.

** Dr. David Dale now at Harwell has since December 1962, been pursuing this suggestion of Peterson and Smith. He has been trying to apply to neutron scattering the method of phasing that the Oxford group (Dale, Hodgkin and Venkatesan[9]) so successfully used in solving the structure of Factor V (I) a.

‡ According to the original Breit-Wigner formula the imaginary part is negative, indicating a phase lag of 90° w.r.t. the real part. However the positive sign is used (phase lead of 90°) in accordance with the experimental result of Peterson and Smith.[7]

This is indeed an achievement as the incident intensity near $\lambda = 0.68\,Å$ is itself quite low and the absorption in this region for Cd^{113} is extremely high.
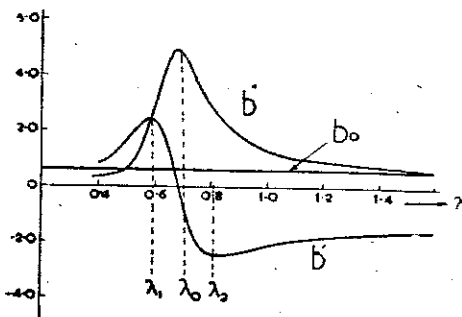

FIG. 2. Variation of b_0, b' and b'' with wavelength near the absorption for Cd^{113}.

Comparing the dispersion correction curves for X-rays and neutrons one finds that $\triangle f'/f_0$ and $\triangle f''/f_0$ have a maximum value of about 0·15 (at $\sin\theta = 0$) and about 0·30 at $\sin\theta = 0.8$ while these ratios for neutrons are almost two orders of magnitude larger, ($b'/b \simeq 5.0\ -7.0$ and $b''/b_0 \simeq 10$ to 12). Further the very forms of the dispersion curves in the two cases are completely different. The imaginary part b'' has a large positive value $(10\,b_0)$ at the resonance wavelength dropping to smaller positive values of the order of b_0 on either side. On the other hand the real part of the scattering factor b' reaches a maximum $(+5\,b_0)$ on the shorter wavelength side of the resonance wavelength, becomes zero at resonance and beyond this becomes negative, reaching a minimum $(-5\,b_0)$ on the longer wavelength side. Peterson and Smith have actually verified this negative scattering predicted by the Breit-Wigner formula. We shall in the next sections examine how these qualitative and quantitative differences between the X-ray and neutron anomalous dispersion could be used effectively for solving the phase problem.

4. THE CONCEPT OF THE "HEAVY ATOM" IN NEUTRON SCATTERING

Normally the neutron scattering lengths of most atoms are of the same order of magnitude. Hence there is nothing corresponding to the "heavy atom" in neutron diffraction. However if the structure contains an anomalous scatterer of neutrons in the asymmetric unit and if a wavelength for which $|b'|$ is large is used, the real part of the scattering factor would be 5

to 10 times the scattering factor of normal atoms. This is equivalent to having a heavy atom in the structure. Techniques used in X-ray diffraction such as the Patterson synthesis could therefore be used for locating these "heavy atoms".

(a) *The Patterson synthesis* : For the pth atom

$$b_p = [(b_0)_p + b_p'] + ib_p'' = B_p' + ib_p''.$$

It can easily be shown that

$$|F(hkl)|^2 = \sum_p \sum_q (B_p' B_q' + b_p'' b_q'')$$
$$\times \cos 2\pi [h(x_p - x_q)$$
$$+ k(y_p - y_q) + l(z_p - z_q)]$$
$$+ \sum_p \sum_q (B_p' b_q'' - B_q b_p'')$$
$$\times \sin 2\pi [h(x_p - x_q)$$
$$+ k(y_p - y_q) + l(z_p - z_q)].$$

Hence the Patterson synthesis

$$P(u, v, w) = \sum_0^\infty h \sum_0^\infty k \sum_0^\infty l |F(hkl)|^2$$
$$\times \cos 2\pi (hu + kv + lw)$$

contains peaks of height $(B_p' B_q' + b_p'' b_q'')$ at $u = x_p - x_q, v = y_p - y_q, w = z_p - z_q$. It must be noted that in neutron scattering unlike in X-ray diffraction we have negative normal scatterers (particularly hydrogen). Thus the positive peaks in the Patterson correspond to interactions between like-scatterers while negative peaks to interaction between unlike-scatterers.

For normal scatterers(N):

$$|B_p'| = |(b_0)_p| > 0, \ b_p' = b_p'' = 0.$$

For anomalous scatterers(A):

$$|B_p'| = |(b_0)_p + b_p'| > 0, \ b_p'' > 0.$$

Hence in the Patterson synthesis the following vectors will be present.

$A_n \to A_m$ of height $(B'_{An} B'_{Am} + b''_{An} b''_{Am})$

$A_n \to N$ of height $(B'_{An} b_N)$

and

$N_n \to N_m$ of height $(b_{Nm} b_{Nn})$

It is quite clear that the interactions between anomalous scatterers will dominate and so it would be possible to identify these peaks. In a specific example if a structure has one Cd atom and a large number of other atoms, the Cd–Cd peak would normally have a height of b_0^2 ($b_0 = 0.4 \times 10^{-12}$). However, if the structure contains Cd^{113} and a wavelength 0.825 Å is used then $b' = - 6 b_0$ and $b'' = + 6 b_0$. Thus $B'_{Cd} = (b_0 - 6 b_0)$ and so the Cd–Cd peak will have a height of $25 b_0^2 + 36 b_0^2 = 61 b_0^2$ while the Cd–N and N–N interactions will have heights

approximately $\mp 5 b_0^2$ and b_0^2 respectively (assuming $|b_0| \simeq |b_N|$).

It is common experience in X-ray crystallography that the heavy atom–heavy atom interactions are swamped out by the heavy atom–light atom vectors if the number of light atoms is sufficiently large. Various techniques have been suggested to overcome this.[12] Neutron anomalous scattering provides an effective method for eliminating the heavy atom–light atom vectors rendering the identification of the heavy atom–heavy atom vectors easy.

If the intensity measurements are made at wavelength λ_0 at which $B_A' = 0$ and $b_A'' > 0$ (Fig. 2) and $B_N' > 0$ and $b_N'' = 0$ then in the Patterson synthesis there will be no A–N vectors and only the A–A vectors (weight $121 b_0^2$) and N–N vectors (weight b_0^2) will survive.

(b) *The Two-Wavelength Method.*—If the measurements are made at two wavelengths on either side of the resonance wavelength then it should be comparatively easy to determine the position of the anomalous scatterer. Let us for the sake of convenience choose two wavelengths λ_1 and λ_2 such that $B'_{\lambda_1} = - B'_{\lambda_2}$ and $b''_{\lambda_1} \simeq b''_{\lambda_2}$. The Patterson syntheses with $|F(hkl)|^2_{\lambda_1}$ and $|F(hkl)|^2_{\lambda_2}$ as coefficients would be similar except that the weights A–N vectors would have changed signs (the A–A and N–N vectors would however all be identical). An addition-Patterson with coefficients $|F(hkl)|^2_{\lambda_1} + |F(hkl)|^2_{\lambda_2}$ would therefore give only the A–A and N–N interactions while the A–N vectors would be suppressed. It must be noted that A–N vectors will not be suppressed completely if b''_{λ_1} and b''_{λ_2} are much different. A single set of measurements of intensities at λ_0 would be as effective as the measurements at two wavelengths, so far as the location of the heavy atom is concerned. However the two-wavelength method has many advantages over the resonant wavelength method such as lower absorption, possibility of determining the phases of reflections uniquely, etc.

A subtraction Patterson with coefficients $|F(hkl)|^2_{\lambda_2} - |F(hkl)|^2_{\lambda_1}$ would only give A–N vectors. Even if B'_{λ_1} is not exactly equal to $- B'_{\lambda_2}$ one could by algebraic manipulations of the data formulate syntheses which give essentially the above results.

PHASE DETERMINATION PROCEDURES

It is clear from the above discussion that one can use the heavy atom technique in neutron diffraction to determine the phases of reflections. In X-ray diffraction, the heavy atom method

is successful in phase determination even when the heavy atom ratio $[\sum f_H{}^2/\sum(f_l{}^2+f_H{}^2)]$ is as low as 0·10 (Vitamin B_{12}). One has only to redefine for anomalous neutron diffraction the heavy atom ratio as $\sum(B_A{}'^2+b_A{}''^2)/\sum(b_N{}^2+B_A{}'^2+b_A{}''^2)$. This ratio is 0·10 even if the asymmetric unit contains only one Cd^{113} and 1,000 to 2,000 normal scatterers and thus the heavy-atom method may succeed. If Sm^{149} or Gd^{157} are used the proportion of the normal scattering atoms can be very much larger.

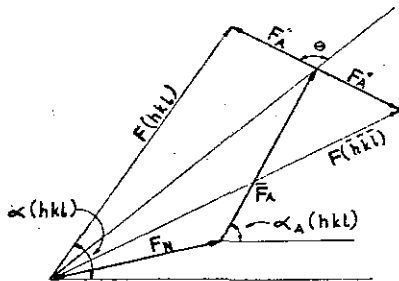By measuring the intensity $I(hkl)$ and

FIG. 3. The vector phase diagram for $F(hkl)$ and $F(\bar{h}\,\bar{k}\bar{l})$.

$I(\bar{h}\bar{k}\bar{l})$ one could get the phase angle (Fig. 3).

$$\sin[\alpha(hkl)-\alpha_A(hkl)]$$
$$=\frac{|F(hkl)|^2-|F(\bar{h}\bar{k}\bar{l})|^2}{4[\frac{1}{2}\{|F(hkl)|^2+|F(\bar{h}\bar{k}\bar{l})|^2\}-F_A''^2]^{\frac{1}{2}}F_A'}$$

If the position of the anomalous scatterer is known then from the methods given above, $\alpha(hkl)$ and F_A'' may be calculated from which $\alpha(hkl)$, the phase of the reflection, could be computed. However, there would be a twofold ambiguity in the phase angle of each reflection. If the anomalous scatterers are not centro-symmetrically distributed, then a Fourier synthesis with both the phases should in principle give the structure. When $b'' \simeq b' \gg b_0$ one must be cautious in applying many of the methods used in X-ray crystallography where $\triangle f'' \simeq \triangle f' \ll f_0$.

Thus a convenient method to determine phases would be to (a) measure the intensities of the Friedel pairs for the two wavelengths λ_1 and λ_2, (b) determine the position of anomalous scatterer by addition—Patterson or its equivalent, (c) calculate α_A from the known position of the anomalous scatterer, and (d) evaluate $\alpha(hkl)$ using $|F(hkl)|_{\lambda_1}$, $|F(\bar{h}\bar{k}\bar{l})|_{\lambda_1}$ and $|F(hkl)|_{\lambda_2}$ in a Harker construction (Fig. 4).

The work of Prof. Dorothy Hodgkin and her collaborators [e.g., Factor VI(a), Vitamin B_{12} mono acid] indicates that with one cobalt ($\triangle f''/f_0 \simeq 0·15$ for CuK_a) atom it is possible
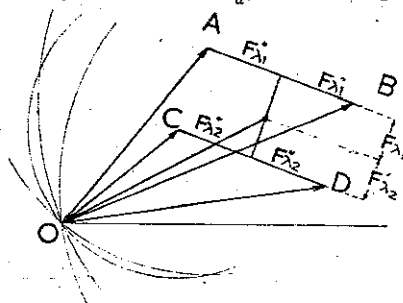

FIG. 4. Method of unique phasing in neutron diffraction $OA=[F(hkl)]_{\lambda_1}$, $OB=[F(\bar{h}\bar{k}\bar{l})]_{\lambda_1}$, $OC=[F(hkl)]_{\lambda_2}$, and $OD=[F(\bar{h}\bar{k}\bar{l})]_{\lambda_2}$.

to solve structures containing about a hundred non-hydrogen light atoms. With the same space group $P2$ it must be possible to phase reflections of a structure containing 10,000 atoms and one Cd^{113} per asymmetric unit. With Sm^{149} and Gd^{157}, the number of light atoms could increase three- or fourfold.

THE CHOICE OF ANOMALOUS SCATTERER AND THE EXPERIMENTAL PROBLEMS

The absorption due to the anomalous scatterer would be inordinately large near the resonant frequency. However, the overall absorption would be much smaller if a large number of light atoms are present. If one wishes to study smaller molecules one could reduce the proportion of the resonant-isotope by diluting it with normal isotope.

The measurement of accurate intensities in neutron scattering in such large structures is fraught with many difficulties. (a) There is a decrease in the intensity due to the large size of the unit cell. (b) The divergence of the incident beam has to be cut down, with a consequent decrease in incident intensity, to resolve the closely spaced reflections. (c) The background intensity due to incoherent scattering is expected to be large. Besides these, one has to grow large single crystals with the resonant-isotope introduced at proper sites.

There are four elements Eu^{151} ($\lambda_0 \simeq 0·6$ Å), Cd^{113}($\lambda_0 \simeq 0·68$ Å), Sm^{149}($\lambda_0 \simeq 0·93$) and Gd^{157}($\lambda_0 \simeq 1·8$ Å) which show resonance in the thermal neutron range. Of these Sm^{149} and Gd^{157} would be perhaps most useful because

their resonance frequencies are close to the maximum of the thermal neutron spectrum of a normal nuclear pile. Further the increase in scattering due to increase in wavelength enhances the intensity of each reflection.

As the signal-to-noise ratio in these crystals of large molecules is expected to be low, one has to explore the possibility of measuring the intensity of a large number of reflections simultaneously (as in the Phillips-technique[13]). One must also think of extending such unconventional methods as spark-chamber techniques for the measurement of intensities to neutron diffraction.

In view of the immense potentialities of the new method for solving very large structures, it may be worthwhile developing experimental methods for measuring the intensities.

1. Coster, D., Knol, K. S. and Prins. J. A., *Z. Phys.*, 1930, **63**, 345.

2. Bijvoet, J. M., *Proc. Acad. Sci., Ams.*, 1949, **52**, 313; Peerdeman, A. F. Bommel, A. J. V. and Bijvoet, J. M., *Ibid.*, 1951, **54**, 16; *Acta Cryst.*, 1956, **9**, 1012.

3. Ramachandran, G. N. and Raman, S., *Curr. Sci., India*, 1956, **25**, 348.

4. Mark, H. and Szilard, L., *Z. Phys.*, 1925, **33**, 688.

5. Ramaseshan, S., Venkatesan, K. and Mani, N. V., *Proc. Ind. Acad Sci.*, 1957, **46 A**, 95; *Curr. Sci.*, **26**, 352.

6. —, *Advanced Methods of Crystallography*, Academic Press, 1964.

7. Peterson, S. W. and Smith, H. G., *Phys Rev.*, 1961, **6**, 7; *Jour. Phys. Soc., Japan*, 1962, **17**, 335; *Jour. de Phys. et la Rad.*, 1962, 191.

8. Johnson, C. K., Cabe, E. J., Taylor, M. R. and Rose, I. A., *Amerchem Soc.*, 1965.

9. Dale, D., Hodgkin, D. C. and Venkatesan, K., *Crystallography and Crystal Perfection*, Academic Press, 1963, p. 237.

10. Breit, G. and Wigner, E., *Phys. Rev.*, 1936, **49**, 519.

11. Brockhouse, B. N., *Can. J. Phys.*, 1953, **31**, 432.

12. Rossmann. M. G., *Acta Cryst.*, 1961, **14**, 383; see Dickerson, R. E., *The Proteins*, Academic Press, N. Y., 1964, **2**; Karth, G. and Parthasarathy, R., *Acta Cryst.*, 1965, **18**, 745; Singh, A. K. and Ramaseshan, S. (under publication).

13. Phillips, D. C. J., *Sci. Instrum.*, 1965.