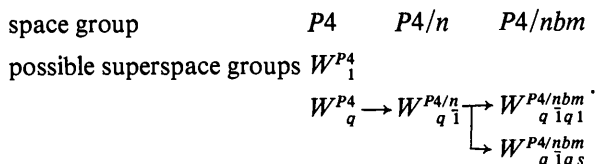$(\frac{1}{2}\frac{1}{2}\gamma)_*$, the translation $\mathbf{a}$ has a t part $\tau_2 = \frac{1}{2}$. Accordingly, (6.1) with $g_1 = (R_1, \varepsilon_1 = 1, \mathbf{s} = 0, \tau_1 = ?)$ and $g_2 = (1, 1, \mathbf{a}, \tau_2 = \frac{1}{2})$ gives

$$\tau_3 = \tau_1 + \tfrac{1}{2}. \qquad (6.4)$$

Since g_1 is of order 4, the possible τ_1 values are 0, $\frac{1}{4}$ or $\frac{1}{2}$ and we have $(\tau_1, \tau_3) = (0, \frac{1}{2})$, $(\frac{1}{2}, 0)$ or $(\frac{1}{4}, -\frac{1}{4})$. The first two are equivalent (superspace group 75.20.1), the last is 75.20.2.

Adding the n mirror with $\varepsilon = -1$, we note that it transforms g_1 and g_3 into each other. By (6.3), this requires $\tau_3 = -\tau_1$, which rules out the $(0, \frac{1}{2})$ case, leaving only $(\frac{1}{4}, -\frac{1}{4})$: 85.20.3. Finally, a mirror plane parallel to the fourfold axes is added to obtain no. 125, for instance the diagonal m plane. From the spatial configuration (m lies between the axes) it follows that m transforms g_1 into g_3^{-1}. According to (6.3) this gives $\tau_3 = -\tau_1' = -\tau_1$, which agrees with the former result independently of the τ assigned to m. Hence, τ can be either 0 (125.20.5) or $\frac{1}{2}$ (125.20.6). Since the mirror b in $P4/nbm$ is the product of m and g_1, (6.1) yields $\tau = \pm\frac{1}{4}$ for b, so the complete pedigree becomes:

space group $P4$ $P4/n$ $P4/nbm$

possible superspace groups W_1^{P4}

$$W_q^{P4} \rightarrow W_{q\,\bar{1}}^{P4/n} \begin{cases} \rightarrow W_{q\,\bar{1}q\,1}^{P4/nbm} \\ \rightarrow W_{q\,\bar{1}q\,s}^{P4/nbm} \end{cases}$$

Of course, different lines of ascendence are possible, such as $P4 - P4bm - P4/nbm$, to arrive at no. 125, which is in the most complicated arithmetic class of all; the derivation of the majority of superspace groups is simpler than in the above example.

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Optimum Choice of Wavelengths in the Anomalous Scattering Technique with Synchrotron Radiation

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Abstract

A formula has been derived for the mean-square error in the phases of crystal reflections determined through the multiwavelength anomalous scattering method. The error is written in terms of a simple function of the positions in the complex plane of the 'centres' corresponding to the different wavelengths. For the case of three centres, the mean-square error is inversely proportional to the area of the triangle formed by them.

The theoretical values are in good agreement with those obtained by earlier workers from computer simulations. The present method makes it easier to optimize the number and the actual wavelengths to be employed in the multiwavelength method. The maximum benefits of this method are expected in experiments employing synchrotron radiation or neutrons.

Introduction

Templeton, Phillips & Hodgson (1980) have recently studied the anomalous scattering of X-rays by caesium with synchrotron radiation (Fig. 1). They have pointed out that the unprecedentedly large anomalous factors near the L-absorption edges ($f' = -26 \cdot 7$ electrons, $f'' = 16 \cdot 1$ electrons) could revive the multiwavelength method (Ramaseshan, 1966; Singh & Ramaseshan, 1968) as a viable alternative to isomorphous replacement for solving the phase problem in macromolecular crystallography. The multiwavelength method with synchrotron radiation has the following attractive features:

(a) One can have as many 'derivatives' as necessary by just tuning to different wavelengths near the absorption edge of the anomalous scatterer.
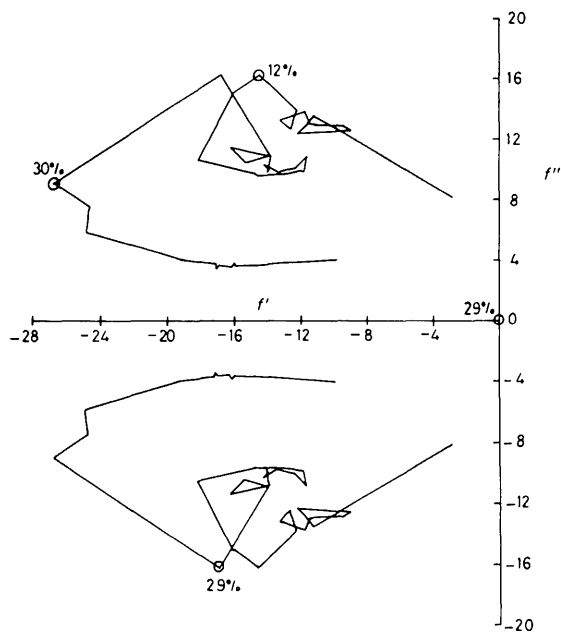

Fig. 1. Anomalous scattering factors for caesium in caesium tartrate near the L_I, L_{II} and L_{III} edges (from Templeton et al., 1980). The contours represent combinations of f' and $\pm f''$ at specific wavelengths. An optimal selection of wavelengths is shown by the four circled points. The points were selected by the methods described in this paper, assuming for simplicity that the source intensity and crystal absorption are the same at all wavelengths. Reflections hkl (top half of the diagram) and $\bar{h}\bar{k}\bar{l}$ (bottom half) must be measured with counting times in proportion to the given percentages.

(b) There is perfect isomorphism since the same crystal is used at all wavelengths.

(c) Owing to the large anomalous scattering factors, the differences between 'derivatives' are almost comparable to those obtained in the isomorphous replacement method, as pointed out by Templeton et al. (1980).

The above features coupled with the flexibility possible suggest that the multiwavelength method with synchrotron radiation is likely to become increasingly competitive with the isomorphous replacement technique. The following questions will, however, have to be answered before the technique can actually be used effectively. (a) How many wavelengths should one use? (b) Which specific wavelengths should be selected? (c) How much experimental time should be allotted to each of the wavelengths?

Phillips & Hodgson (1980) have made a start in answering the first two questions. With a computer simulation approach, they calculate the expected r.m.s. error in the phase determination for each promising set of wavelengths. They suggest that the best set can thus be selected. This method is quite time consuming. In this paper, we present an analytical theory from which we derive a simple *formula* for the r.m.s. phase error, expressed in terms of the coordinates in the complex plane of the 'centres' corresponding to the different wavelengths. The present approach is simpler than that of Phillips & Hodgson and we believe it can be routinely used to answer the above questions.

Theory

In the following, we assume that the positions of the anomalous scatterers in the (protein) structure have already been obtained by means of one of the various well known methods (e.g. Kartha, 1975). We are concerned with the next stage, viz the determination of the phases of the reflections. These are obtained by the method of Blow & Crick (1959) or its many modifications (Cullis, Muirhead, Perutz, Rossmann & North, 1961; North, 1965; Matthews, 1966) from intensity measurements corresponding to a number of isomorphous derivatives (or different wavelengths in the multiwavelength method). In this paper we consider the following scheme, which is very close to the one suggested and used by Cullis et al. (1961). We are given n 'centres' (such as P, A, B in Fig. 2) having measured structure-factor amplitudes F_i with r.m.s. errors* Δ_i. Circles of radii F_i are drawn around each of

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* The r.m.s. errors corresponding to different centres would in general be different because of (a) different intensities of the radiation source, (b) different absorptions of the crystal and (c) different measuring times. Of these, (c) could be adjusted by the experimenter, as discussed later.

the centres. The phase of the reflection is then determined as follows:

(a) Associate weights $1/\Delta_i^2$ with the n centres and calculate their 'centre of mass'. From this point draw radius vectors in various directions.

(b) In each direction, determine the intersections of the n circles with the radius vector and compute their weighted variance.

(c) The radius vector having the minimum variance is selected to estimate the phase φ_P of the reflection.

To analyse the r.m.s. error between the estimated phase and the true phase, we make the following further simplifying assumptions:*

(a) The centres cluster within an area whose linear dimensions are much smaller than the radii of the circles. In the language of Fig. 2, the triangle PAB is assumed to be much smaller than the three circles.

(b) The r.m.s. phase error is small compared to 2π.

(c) Because of the above two simplifications, the circles can be replaced by straight lines within the region of interest.

The geometry of the problem is now described in Fig. 3. For convenience we have shown only three centres A, B, C. O' is the centre of mass of the centres. The dashed lines are the 'true' circles corresponding to the case when there are no errors in the radii − these lines intersect at the true origin O. The full lines in Fig. 3 represent the circles when there are errors in the radii. We take coordinate axes with the y axis along $O'O$ and the x axis perpendicular to it at O.

The theory proceeds as follows:†

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* The next section suggests how the theory may be extended to more 'real' situations.

* The details have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36025 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.
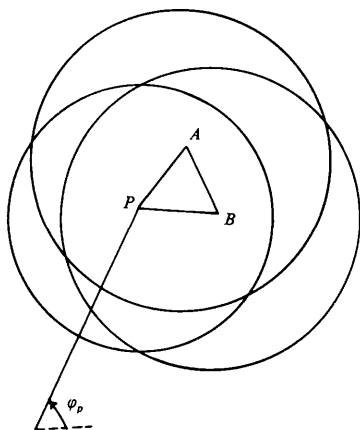
(a) The phase error is obtained for a particular set of errors in the F_i.

(b) It is statistically averaged assuming that the errors on different F_i are uncorrelated and that their r.m.s. values are Δ_i.

(c) This result is circularly averaged for all values of the phase of the reflection.

One then obtains the following expression for the mean-square error (in rad^2) in the phase determination, where (X_i, Y_i) are the coordinates in the complex plane of the ith centre.

$$\langle (\Delta\varphi)^2 \rangle_{\text{calc}} = D^{-1/2} \tag{1}$$

$$D = \left[\sum_{i=1}^{b} \frac{1}{\Delta_i^2} (X_i - \bar{X})^2 \right] \left[\sum_{i=1}^{n} \frac{1}{\Delta_i^2} (Y_i - \bar{Y})^2 \right]$$

$$- \left[\sum_{i=1}^{n} \frac{1}{\Delta_i^2} (X_i - \bar{X})(Y_i - \bar{Y}) \right]^2 \tag{2}$$

$$\bar{X} = \sum_{i=1}^{n} \frac{X_i}{\Delta_i^2} \Big/ \sum_{i=1}^{n} \frac{1}{\Delta_i^2} \tag{3}$$

$$\bar{Y} = \sum_{i=1}^{n} \frac{Y_i}{\Delta_i^2} \Big/ \sum_{i=1}^{n} \frac{1}{\Delta_i^2}. \tag{4}$$

If we associate 'masses' $1/\Delta_i^2$ with the centres, then D is the determinant of the moment-of-inertia tensor of the two-dimensional collection of n 'massy' points. It is therefore invariant with respect to translations and rotations of the coordinate axes. Now, we can write the scattering factor $\mathbf{F}_H$ of the anomalous scatterers alone in the form

$$\mathbf{F}_{Hi}(h,k,l) = \mathbf{S}_H(h,k,l)\,(f_0 + f'_i + if''_i), \tag{5}$$

Fig. 2. Geometry of the method of phase determination. P, A, B are three centres corresponding to different wavelengths and φ_P is the phase of the reflection corresponding to P. The circles have centres at P, A, B and have radii given by the corresponding F_i.
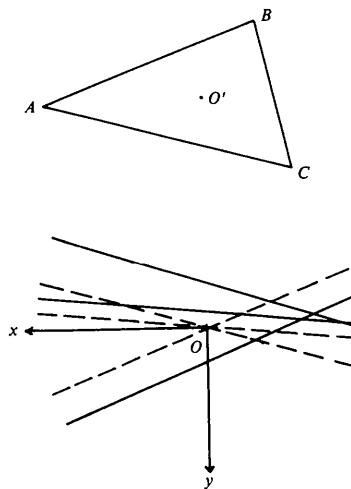

Fig. 3. The simplified geometry of phase determination used in the development of the theory. The significance of the lines is described in the text.

where S_H is structure dependent, f_0 is the scattering factor per anomalous scatterer in the absence of anomalous scattering and f'_i and f''_i are the anomalous factors corresponding to the ith centre. It is then very convenient to make the following choice for X_i and Y_i in (1) to (4).

$$X_i = f'_i |S_H(h,k,l)| \tag{6}$$

$$Y_i = f''_i |S_H(h,k,l)|. \tag{7}$$

It should be pointed out that $|S_H|$ enters only as a scale factor in (X_i, Y_i). Therefore, although it will determine the actual magnitude of $\langle (\Delta\varphi)^2 \rangle_{calc}$ in (1), it is irrelevant for much of the later discussion where one is concerned with the *relative* accuracies achievable with different sets of centres. Another interesting feature of (1) to (4) is that the F_i themselves have completely dropped out and it is only the Δ_i that are important.

Expression (2) [with (6) and (7)] can be simplified in a few special cases:

(a) When there are only three centres and their r.m.s. errors are equal, then

$$D = \frac{4}{3} \frac{|S_H|^4}{\Delta^4} A^2, \tag{8}$$

where A, the area of the triangle formed by the three points, is given by

$$A = f'_1(f''_2 - f''_3) + f'_2(f''_3 - f''_1) + f'_3(f''_1 - f''_2). \tag{9}$$

Thus, for three centres, one can minimize errors in the phases by maximizing the area of the triangle formed by them.

(b) When one employs m wavelengths and measures Friedel pairs at each (a total of $2m$ centres), then, for equal r.m.s. errors at the $2m$ centres, one has

$$D = \frac{4|S_H|^4}{\Delta^4} \left[\sum_{i=1}^{m} (f'_i - \bar{f}')^2 \right] \left[\sum_{i=1}^{m} f''^2_i \right]. \tag{10}$$

In particular, for two wavelengths and four centres,

$$D = \frac{2|S_H|^4}{\Delta^4} (f'_1 - f'_2)^2 (f''^2_1 + f''^2_2). \tag{11}$$

Verification and empirical extension of the theory

We compare our theoretical results with the detailed results obtained by Phillips & Hodgson (1980; see also Phillips, 1978) with a computer simulation approach. They have considered a hypothetical protein with caesium atoms on which computer 'experiments' are carried out at specified wavelengths at the L edges of caesium. They have limited themselves to a basic set of twelve centres (Fig. 4 shows the values of f' and f'').

Out of these they have considered ten different experiments ranging from sets of three centres up to ten centres (Table 1). For each set, they have obtained the phase errors between the calculated and true values for 1000 reflections. They have then listed the r.m.s. phase errors for each set as a function of σ, the r.m.s. error in the simulated measured intensities (assumed to be the same for all the centres), and N, the molecular weight of the simulated protein. For comparison purposes, we have normalized and consolidated the values of Phillips & Hodgson to a standard case corresponding to $\sigma = 1\%$ and $N = 12\,000$. In doing this, we have considered only those of their results which have r.m.s. phase errors $\lesssim 10°$ since our theory is essentially valid only in the limit of small phase errors.[*] The normalized results of Phillips & Hodgson are listed in Table 1.

According to our theory, for constant σ and N, the r.m.s. phase errors should be proportional to $D^{-1/4}$. We have calculated D for all the ten sets of centres with the simplified formulae (8)–(11) taking $\Delta/|S_H|$ to be 0·912 electrons. The close agreement between our calculated r.m.s phase errors and the 'experimental' results of Phillips & Hodgson (Table 1) verifies the essential correctness of our theory. The present calculations are, of course, much simpler to implement than the earlier computer simulation approach.

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[*] However, note the extension of the theory discussed later.
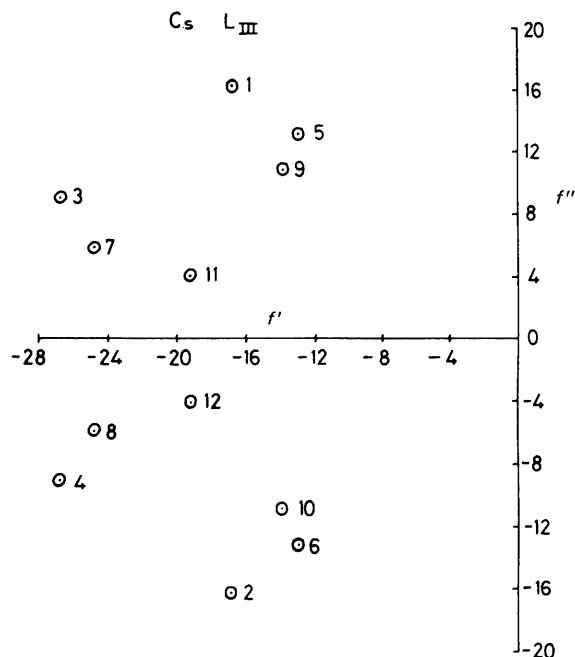

Fig. 4. Twelve centres selected by Phillips & Hodgson (1980) near the L_{III} edge of caesium in caesium tartrate. The theory developed here is compared with the computer simulation results of Phillips & Hodgson on the above centres.

Table 1. *R.m.s. phase errors in a hypothetical protein crystal for various choices of centres*

Comparison of the values of Phillips & Hodgson (1980) with those calculated with the present theory.

Combination of centres in Fig. 4	R.m.s. phase error (°)		Relative experimental time for constant phase error
	Phillips & Hodgson (1980) average of values $\lesssim 10°$	$D^{-\frac{1}{4}}$	
1, 2, 3	6·09	5·97	118·5
1, 2, 3, 4	5·20	5·17	118·5
3, 5, 6	5·62	5·58	103·5
3, 4, 5, 6	4·65	4·75	100·0
1, 2, 3, 4, 5, 6	3·80	3·91	101·6
2, 3, 5	5·38	5·56	102·8
1, 2, 3, 4, 5, 6, 7, 8	3·57	3·63	116·8
1, 2, 3, 4, 5, 6, 9, 10	3·50	3·56	112·3
1, 2, 3, 4, 5, 6, 7, 8, 9, 10	3·29	3·26	117·8
1, 3, 11	10·7	10·47	364·4

The theoretical results of the previous sections are valid in the limit of small phase errors and may be expected to become increasingly incorrect at larger errors. For instance, as $D \to 0$, $\langle(\Delta\varphi)^2\rangle_{calc} \to \infty$. On the other hand, phase being a circularly periodic quantity, the true mean-square phase error $\langle(\Delta\varphi)^2\rangle_{true} \to \pi^2/3$. However, it is reasonable to suppose that $\langle(\Delta\varphi)^2\rangle_{true}$ and $\langle(\Delta\varphi)^2\rangle_{calc}$ are approximately related by a simple monotonic relation. That there *is* such a relationship is shown by Fig. 5 which plots the r.m.s. phase errors, true (*i.e.* given by Phillips & Hodgson, 1980) *versus* calculated. The existence of a monotonic one-to-one relationship between $\langle(\Delta\varphi)^2\rangle_{true}$ and $\langle(\Delta\varphi)^2\rangle_{calc}$ greatly increases the usefulness of the present theory. Thus, even when the phase errors are large, $\langle(\Delta\varphi)^2\rangle_{calc}$ can still be used to decide the best combination of centres (*i.e.* the optimum wavelengths).

In some situations it might be important to be able to estimate $\langle(\Delta\varphi)^2\rangle_{true}$ itself. One then needs a quantitative relation between $\langle(\Delta\varphi)^2\rangle_{true}$ and $\langle(\Delta\varphi)^2\rangle_{calc}$. We have fitted the points in Fig. 5 and obtained the following empirical equation:

$$\langle(\Delta\varphi)^2\rangle_{true}^{1/2} = \langle(\Delta\varphi)^2\rangle_{calc}^{1/2}/[1 + 0\cdot46 \langle(\Delta\varphi)^2\rangle_{calc}^{0\cdot65}]^{(1/1\cdot30)}, \quad (12)$$

where all angles are measured in rad. The above relation has the property that $\langle(\Delta\varphi)^2\rangle_{true}^{1/2} \simeq \langle(\Delta\varphi)^2\rangle_{calc}^{1/2}$ for small phase errors and asymptotically tends to the value $\pi/\sqrt{3}$ for large errors. Equation (12) is frankly empirical but is nevertheless likely to be useful.

Discussion of strategy for protein crystallography

With the help of the theoretical results developed in this paper it is possible to design and plan protein crystallography experiments carefully. We are primarily concerned here with experiments with synchrotron X-radiation or neutrons, where one is able to

change the wavelength at will and thus select suitable combinations of the anomalous factors. The strategy *par excellence* would be to optimize simultaneously both the wavelengths and the experimental times allotted to the wavelengths. One should choose different promising combinations of wavelengths and in each case optimize the relative times so as to maximize D. One can then select the best set of wavelengths. The optimization of the relative times allotted to the different wavelengths leads to equations which are, unfortunately, non-linear and which will have to be solved iteratively. However, very high convergence will not be required since the r.m.s. phase errors depend only on $D^{-1/4}$ which will be nearly constant for quite large deviations of the relative times from the optimum values.

A point worth remembering is that the wavelengths of neutrons or synchrotron radiation are so easily tuned that it is not necessary to limit oneself to Friedel pairs. Thus if one has decided to use four centres, then it may be worthwhile looking for four different wavelengths rather than for Friedel pairs at two wavelengths. For instance, with the data of Fig. 1 on caesium, we have determined the best combination of four centres under the assumption of constant source intensity and absorption at all wavelengths. We obtain the results shown in Fig. 1. The optimum relative times for the centres are 30, 12, 29 and 29% of the total available time. We note that there are no Friedel pairs at all. In the neutron case, for a single-term Breit–Wigner-type resonance (Taylor, 1972), the best strategy is to measure Friedel pairs at two symmetrical wavelengths as shown in Fig. 6. In this case, by symmetry, all the four centres should be given equal experimental times.

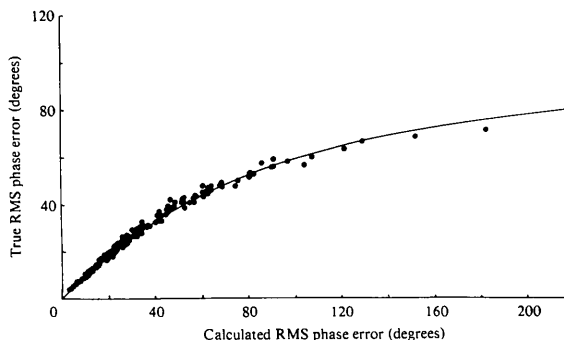The optimization of relative times for different wavelengths is somewhat tedious and probably not

Fig. 5. True r.m.s. phase errors as obtained by Phillips & Hodgson (1980) plotted against the values calculated by means of the present theory. The points show strong evidence for the existence of a monotonic one-to-one relation between the two quantities. The solid line represents the empirical relation described by equation (12).

necessary for many experiments since the potential gains are not very great. However, if one decides to give equal times (or equal counts) to all the centres, the choice of the optimum set of centres is a very simple matter since the quantity D (equation 2) which is to be maximized can be easily calculated. We strongly feel that this minimum amount of optimization should always be carried out before using the multiwavelength method.

Regarding the question of how many centres to select, the answer obviously varies from case to case. However, our experience with the data of Fig. 1 suggests that three or four centres give the best accuracy of phase determination for a given total experimental time. This is substantiated by Table 1 where we have listed the relative experimental times needed with the various sets of centres in order to
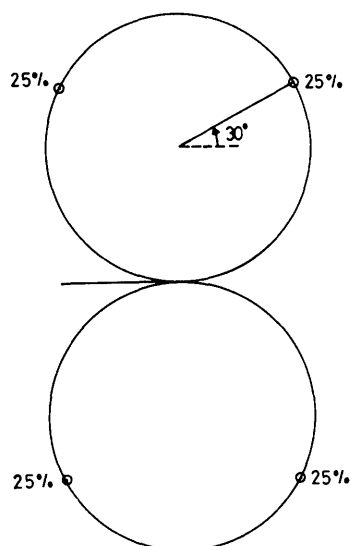
achieve the same r.m.s. phase error. Incidentally, these numbers suggest that there could well be a 20% difference in efficiency between two sets of centres which may look equally good on simple inspection. On the question of the optimum number of centres, we should caution that all the results of this paper presuppose exact knowledge of the positions of the heavy atoms as well as the absence of other systematic errors. In 'real' situations, there is safety in redundant information, and it would probably be preferable to have a larger number of centres.

6. Conclusion

We have derived in this paper an expression for the r.m.s error in the phases of reflections estimated by the multiwavelength method. With this expression we have suggested means of optimizing such experiments. The computations involved are relatively straightforward. We believe that the optimization procedures suggested here can be advantageously used, especially in protein crystallography.

Fig. 6. Neutron anomalous scattering factors near resonance of a nucleus describable by a single-term Breit–Wigner theory. The two circles represent on the Argand diagram the values of $f_0 + f'$ $\pm$ if'' at various neutron wavelengths. The present theory suggests that the optimal neutron wavelengths to be employed in the multiwavelength method are those corresponding to the four circled points. Identical measuring times are recommended for all the centres.

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