The correlation coefficient r of the pair $|E_{\mathbf{H}}|^2$, $|G_{\mathbf{H}}|^2$,

$$r=\langle(|E_{\mathbf{H}}|^2-\overline{|E_{\mathbf{H}}|^2})(|G_{\mathbf{H}}|^2-\overline{|G_{\mathbf{H}}|^2})\rangle_{\mathbf{H}} \times\langle(|E_{\mathbf{H}}|^2-\overline{|E_{\mathbf{H}}|^2})^2\rangle_{\mathbf{H}}^{-1/2}\langle(|G_{\mathbf{H}}|^2-\overline{|G_{\mathbf{H}}|^2})^2\rangle_{\mathbf{H}}^{-1/2} \qquad (25)$$

is shown to be equal to $\alpha_{11}^2/\alpha_{20}\alpha_{02}$. Under the assumption that the atomic content of the derivative equals the atomic content of the native protein (p) plus the heavy-atom content (H),

$$r=1/(1+\sum Z_H^2/\sum Z_p^2) = 1/[1+(\text{diffraction ratio})^2/2]. \qquad (26)$$

2.5. *Phase accessibility*

As is the case of the traditional three-phase invariant, although probability distributions can be calculated for the whole family of invariants (at a given resolution) only a subset of these invariants can be reliably estimated. In the present case, however, this subset consists largely of invariants whose phases are associated with normalized structure factors for which large differences between the native and derivative diffraction intensities are observed. Consequently only a subset of the structure factors can be reliably phased. Unfortunately, this subset, in some cases, may not coincide with that needed to calculate an interpretable density map. It is hoped that, in those instances, the phase set may be extended through the use of quartet invariants, standard phase extension techniques or density modification procedures.

3. Concluding remarks

In recent years, a formal mathematical integration of the techniques of direct methods and isomorphous replacement has been undertaken (Hauptman, 1982; Fortier, Weeks & Hauptman, 1984). The amount of information contained in the probability distributions is extensive, although often hidden behind the mathematical complexity of the formulae. As we have shown, it is relatively easy to translate the distributions into the usual experimental parameters. Through such an exercise, a better understanding of the nature and scope of the distributions is attained. Conversely, the mathematical formulae yield a better understanding of the experiment, and indicate ways to improve and gauge the experiment. The exact role of direct methods in macromolecular structure determination cannot be predicted at this point. As has been the case with the traditional direct methods, several years of experience in the application of these methods will probably be needed before an accurate evaluation can be made. With the extensive theoretical base now at hand, and the extremely promising results obtained to date, we are now in a position to address many of the unanswered questions, principally pertaining to the application of the methods to real diffraction data.

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Measurability of Bijvoet Differences in Triclinic, Monoclinic and Orthorhombic Crystals

BY D. VELMURUGAN AND S. PARTHASARATHY

*Department of Crystallography and Biophysics,** *University of Madras, Guindy Campus, Madras*-600 025, *India*

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Abstract

Theoretical expressions for the measurability of Bijvoet differences have been derived for triclinic, monoclinic and orthorhombic crystals containing p (= 1 or 2) anomalous scatterers and many normal scatterers per asymmetric unit. Results for the many-atom cases (*i.e.* $P = MN$ and MC cases) in space group $P1$ are also obtained. The theory takes into consideration the effect of data truncation due to unobserved reflections. The measurability values for the various cases are given in the form of compact

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tables as functions of the relevant parameters k and σ_1^2. The theoretical results are also used to discuss briefly the following aspects. (i) The effect of k and σ_1^2 on the measurability. (ii) Measurability to be expected in the heavy-atom derivatives of proteins with Cu $K\alpha$ radiation. (iii) A comparison of the measurabilities in Sm derivatives of a protein for Cu $K\alpha$ and for a wavelength ($\lambda = 1{\cdot}8448$ Å) at which the anomalous scattering effect has been observed to be the largest. It is also found that in the case of the heavy-atom derivatives of proteins measurability is strongly influenced by variation in σ_1^2.

1. Introduction

It is well known that X-ray anomalous scattering (AS, hereafter) provides a powerful method of tackling the phase problem in non-centrosymmetric crystals containing suitable heavy atoms (Ramachandran & Raman, 1956; Peerdeman & Bijvoet, 1956). The power of the AS method for determining complex structures like proteins has been pointed out by Ramachandran & Parthasarathy (1965). The successful structure determination of the macromolecule Flavodoxin (MW 16 000) by the AS method is a good example for the power of this method (see Watenpaugh, Sieker & Jensen, 1975). The advent of synchrotron radiation as a source of radiation for diffraction work in recent years has renewed our interest in the AS method of determining structures of proteins as well as molecules of moderate complexity containing hundreds of atoms. The success of the AS method of structure determination strongly depends on the measurability of Bijvoet differences (*i.e.* on the possibility of measuring fairly accurately the Bijvoet differences of a large percentage of reflections). Problems related to the measurability of Bijvoet differences* such as the optimum conditions, the effect of the number of anomalous scatterers in the unit cell of a triclinic crystal, the effect of space-group symmetry *etc.* have been studied by making use of the probability distributions of the normalized Bijvoet difference (BD, hereafter) variables x and Δ and the modified Bijvoet ratio (BR, hereafter) δ [see equations (6)–(9) for a definition of these variables and see Parthasarathy (1982) for the latest review]. In these papers it has been tacitly assumed that the intensities of all the theoretically possible reflections within a given ($\sin\theta/\lambda$) range could be measured. Numerical values for the measurability have so far been obtained only for triclinic crystals containing one or many† anomalous scatterers in the unit cell (Parthasarathy & Ponnuswamy, 1981*a*) and these values are, however, approximate since they were obtained from a theoretical treatment of the modified BR δ rather than the BR X.‡ Further, the theoretical values for the measurability are not available in the literature for space groups of higher symmetry. In this paper we shall therefore obtain theoretical values of the measurability for crystals of the triclinic, monoclinic and orthorhombic space groups containing one or two anomalous scatterers per asymmetric unit. We shall also derive results for the many-atom cases (*i.e.* MN and MC cases) of space group $P1$. In the present paper the effect of data truncation arising due to non-observability of extremely weak reflections is also taken into account.

The notation used and the preliminary results required in this paper are given in § 2. A quantitative definition of the measurability is given in § 3. Theoretical expressions for the measurability for the various cases are derived in § 4. § 5 contains a brief discussion of the results obtained. Test of the theoretical results is considered in § 6.

2. Notation and preliminary results

Consider a non-centrosymmetric crystal containing N atoms in the unit cell. Let P of these be anomalous scatterers of the same type and Q $(= N - P)$ be normal scatterers of similar scattering power (*e.g.* C, N and O). Let s be the number of equivalent general positions in the unit cell. Let n $(= N/s)$, p $(= P/s)$ and q $(= Q/s)$ be the number of N, P and Q atoms per asymmetric unit. The structure-factor equation for reflection **H** can be written as (see Fig. 1)

$$F_N(\mathbf{H}) = F'_P(\mathbf{H}) + F_Q(\mathbf{H}) + F''_P(\mathbf{H}) = F'_N(\mathbf{H}) + F''_P(\mathbf{H}), \qquad (1)$$

where

$$F'_N(\mathbf{H}) = F'_P(\mathbf{H}) + F_Q(\mathbf{H}). \qquad (2)$$

BD ΔI and the mean intensity $\bar{I}$ of the Bijvoet pair $\mathbf{H}$ $(= hkl)$ and $\bar{\mathbf{H}}$ $(= \bar{h}\bar{k}\bar{l})$ can be written as (Srinivasan & Parthasarathy, 1976)

$$|\Delta I| = 4|F'_N||F''_P||\sin\theta| \qquad (3)$$

$$\bar{I} = |F'_N|^2 + |F''_P|^2, \qquad (4)$$

where

$$\theta = \alpha'_N(\mathbf{H}) - \alpha'_P(\mathbf{H}). \qquad (5)$$

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* In this paper, for brevity, we shall use the term measurability to stand for the measurability of Bijvoet differences of a crystal.

† When there are many anomalous scatterers in the unit cell of a crystal of space group $P1$, these atoms could take up either a centrosymmetric or a non-centrosymmetric configuration. These two situations will be designated as $P = MC$ and $P = MN$ cases, respectively.

‡ A recent theoretical calculation of $\langle X\rangle$ for crystals of space group $P1$ has shown that the value of $\langle X\rangle$, for given k and σ_1^2, is significantly lower than the value of $\langle\delta\rangle$ for the corresponding situation particularly under pronounced AS effect (Baskaran, Parthasarathy & Velmurugan, 1982). For further details see § 5.1.

The BR variables X and δ and the BD variables x and Δ are defined by (Srinivasan and Parthasarathy, 1976)

$$X = |\Delta I|/\bar{I} = \frac{4|F'_N||F''_P||\sin\theta|}{|F'_N|^2+|F''_P|^2} \tag{6}$$

$$\delta = |\Delta I|/|F'_N|^2 = 4|F''_P||\sin\theta|/|F'_N| \tag{7}$$

$$x = \frac{|\Delta I|}{4[\langle|F_Q|^2\rangle\langle|F''_P|^2\rangle]^{1/2}} \tag{8}$$

$$\Delta = |\Delta I|/\langle|F'_N|^2\rangle. \tag{9}$$

Equation (7) is obtained from (6) by neglecting $|F''_P|^2$ in the denominator of (6) in comparison with $|F'_N|^2$. We shall denote a particular value of X by X_0.

The normalized intensities and normalized structure factor magnitudes of the inverse reflections $\mathbf{H}$ and $\bar{\mathbf{H}}$ are defined to be

$$\begin{aligned} z(\mathbf{H}) &= [y(\mathbf{H})]^2 = |F_N(\mathbf{H})|^2/\langle\bar{I}\rangle, \\ z(\bar{\mathbf{H}}) &= [y(\bar{\mathbf{H}})]^2 = |F_N(\bar{\mathbf{H}})|^2/\langle\bar{I}\rangle, \end{aligned} \tag{10}$$

where [see (4)]

$$\langle\bar{I}\rangle = \langle|F'_N|^2\rangle + \langle|F''_P|^2\rangle. \tag{11}$$

Let $z_{\min}$ be the minimum of the values $z(\mathbf{H})$ and $z(\bar{\mathbf{H}})$ and $y_{\min}$ be that of $y(\mathbf{H})$ and $y(\bar{\mathbf{H}})$. Let y_t and z_t denote the threshold values of the normalized structure factor magnitude and that of the normalized intensity, respectively. It is evident that

$$z_{\min} = y^2_{\min} \tag{12}$$

and

$$z_t = y_t^2. \tag{13}$$

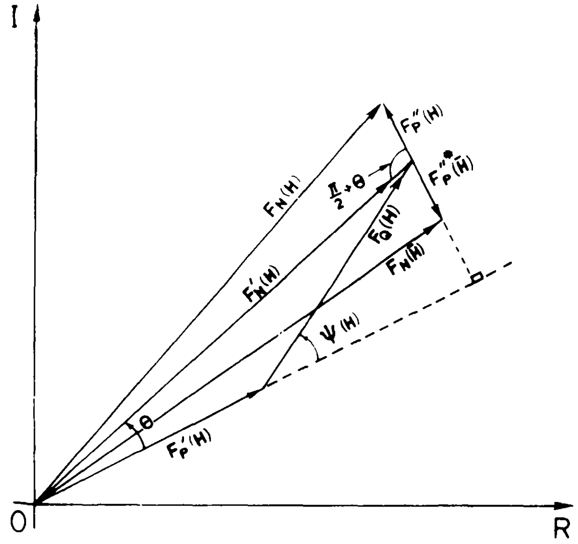

Fig. 1. The relationship between the various components of the structure factors of the inverse reflections $\mathbf{H}$ and $\bar{\mathbf{H}}$ in the presence of anomalous scattering.

For a given crystal, the measurability depends on the parameters k and σ_1^2 which are defined by

$$k = f''_P/(f^0_P + f'_P) \tag{14}$$

and*

$$\sigma_1^2 = \langle|F'_P|^2\rangle/\langle|F'_N|^2\rangle. \tag{15}$$

Here f^0_P is the high-frequency limit of the scattering factor and f'_P and f''_P are the real and imaginary dispersion corrections. The normalized structure factor magnitudes y_Q and y_P† are

$$\begin{aligned} y_Q &= [|F_Q|^2/\langle|F_Q|^2\rangle]^{1/2}, \\ y_P &= [|F'_P|^2/\langle|F'_P|^2\rangle]^{1/2}. \end{aligned} \tag{16}$$

Let $\psi(\mathbf{H})$ be the angle defined by

$$\psi(\mathbf{H}) = \alpha_Q(\mathbf{H}) - \alpha'_P(\mathbf{H}). \tag{17}$$

It follows that

$$\psi(\bar{\mathbf{H}}) = -\psi(\mathbf{H}). \tag{18}$$

In crystals with a single species of anomalous scatterer we have‡ (Srinivasan & Parthasarathy, 1976)

$$|F''_P| = k|F'_P| \tag{19}$$

$$|F'_N|\sin\theta = |F_Q|\sin\psi. \tag{20}$$

Foster & Hargreaves (1963*a*, *b*) have shown that the triclinic, monoclinic and orthorhombic space groups (*Fddd* and *Fdd*2 being the exceptions) can be classified into seven categories based on the trigonometric factors of the geometrical structure factor. Among these, the categories 1, 3, 5 and 6 correspond to the non-centrosymmetric case and hence these alone are of interest in our present study. y_P for these four space-group categories can be written as [see (9) of Parthasarathy & Ponnuswamy, 1976]

$$y_P = E_P/(\varepsilon p)^{1/2}, \tag{21}$$

where ε is 1, $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{4}$ for the categories 1, 3, 5 and 6, respectively, and E_P is given by¶

$$E_P = \left[\left(\sum_{j=1}^{p}\xi_{pj}\right)^2 + \left(\sum_{j=1}^{p}\eta_{pj}\right)^2\right]^{1/2}. \tag{22}$$

Here ξ_{pj} and η_{pj} are the trigonometric parts (excluding the symmetry number) of the structure factor expression listed in *International Tables for X-ray Crystallography* (1969).

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* We shall denote $\langle|F_Q|^2\rangle/\langle|F'_N|^2\rangle$ by σ_2^2. From (2) it follows that $\sigma_1^2+\sigma_2^2=1$.

† Though this should be denoted by y'_P, we shall denote it by y_P for convenience. Since $|F'_P(\mathbf{H})| = |F'_P(\bar{\mathbf{H}})|$, the explicit use of the indices $\mathbf{H}$ and $\bar{\mathbf{H}}$ is superfluous when dealing with these magnitudes. We shall therefore denote these by $|F'_P|$. For similar reasons we shall denote $|F'_N(\mathbf{H})|$ and $|F'_N(\bar{\mathbf{H}})|$ by $|F'_N|$, $|F_Q(\mathbf{H})|$ and $|F_Q(\bar{\mathbf{H}})|$ by $|F_Q|$ and $|F''_P(\mathbf{H})|$ and $|F''_P(\bar{\mathbf{H}})|$ by $|F''_P|$.

‡ Hereafter, for convenience we shall denote $\psi(\mathbf{H})$ by ψ.

¶ The subscripts pj stand for the contribution arising from atom j of the P type (*i.e.* anomalous scatterer) in the asymmetric unit.

3. Definition of the measurability of BDs of a crystal

A BR which is not too small (say $X \geq 0{\cdot}1$) can be easily measured fairly accurately provided the intensity of the reflection **H** and that of the inverse reflection $\bar{\mathbf{H}}$ are both above the background level. Measurability of BDs of a crystal can therefore be quantitatively defined as the fractional number of reflections which satisfy the following two conditions simultaneously:

(i) $X \geq X_0 \, (=0{\cdot}1, \text{say})$

and

(ii) $z_{\min} \geq z_t$

[equivalently, $y_{\min} \geq y_t$; see (12) and (13)]. Using the set-theoretic concept of intersection (denoted by the symbol $\cap$) the simultaneous satisfaction of conditions (i) and (ii) can be written as $[(X \geq X_0) \cap (y_{\min} \geq y_t)]$. For convenience we shall denote the fractional number of reflections satisfying these two conditions by the symbol $M(X_0, y_t)$. Using the probability concept we can therefore write

$$M(X_0, y_t) = \Pr[(X \geq X_0) \cap (y_{\min} \geq y_t)]. \quad (23)$$

It is thus seen that the measurability of BDs for a given crystallographic situation can be obtained by evaluating the probability function on the right-hand side of (23). We shall presently consider this aspect.

4. Derivation of the theoretical expression for $M(X_0, y_t)$

4.1. *Results for cases $p=1$ and 2 in triclinic, monoclinic and orthorhombic space groups*

From (5) we obtain (see Fig. 1)

$$|F'_N|^2 = |F'_P|^2 + |F_Q|^2 + 2|F'_P||F_Q| \cos \psi. \quad (24)$$

Making use of (19) and (20) in the numerator of (6) and (19) and (24) in the denominator of (6), we obtain

$$X = 4k|F'_P||F_Q||\sin \psi| \times [(1+k^2)|F'_P|^2 + |F_Q|^2 + 2|F'_P||F_Q| \cos \psi]^{-1}. \quad (25)$$

In terms of the normalized variables y_P and y_Q [see (16)] we can rewrite (25) as

$$X = 4k\sigma_1\sigma_2 y_P y_Q |\sin \psi| \times [(1+k^2)\sigma_1^2 y_P^2 + \sigma_2^2 y_Q^2 + 2\sigma_1\sigma_2 y_P y_Q \cos \psi]^{-1}. \quad (26)$$

Substituting for y_P from (21) we can rewrite (26) as

$$X = C_1 E_P y_Q |\sin(2\pi\psi_0)| \times [C_2 E_P^2 + \sigma_2^2 y_Q^2 + C_3 E_P y_Q \cos(2\pi\psi_0)]^{-1}, \quad (27)$$

where ψ_0 is defined by

$$\psi_0 = \psi/2\pi \quad (28)$$

and C_1, C_2 and C_3 are defined to be

$$C_1 = 4k\sigma_1\sigma_2/(\varepsilon p)^{1/2},$$
$$C_2 = (1+k^2)\sigma_1^2/(\varepsilon p),$$
$$C_3 = 2\sigma_1\sigma_2/(\varepsilon p)^{1/2}. \quad (29)$$

From Fig. 1 it is seen that

$$|F_N(\mathbf{H})|^2 = |F'_N|^2 + |F''_P|^2 - 2|F'_N||F''_P| \cos\left(\frac{\pi}{2} + \theta\right). \quad (30)$$

Making use of (24) in the first term on the right hand side of (30) and then using (19), (20) and (28), we can rewrite (30) as

$$|F_N(\mathbf{H})|^2 = (1+k^2)|F'_P|^2 + |F_Q|^2 + 2|F'_P||F_Q|(\cos 2\pi\psi_0 + k \sin 2\pi\psi_0). \quad (31)$$

In view of (15) and (19) we obtain from (11)

$$\langle \bar{I} \rangle = (1+k^2\sigma_1^2)\langle |F'_N|^2 \rangle. \quad (32)$$

From (31) and (32) we obtain $z(\mathbf{H})$ to be [see (10)]

$$z(\mathbf{H}) = [(1+k^2)|F'_P|^2 + |F_Q|^2 + 2|F'_P||F_Q|(\cos 2\pi\psi_0 + k \sin 2\pi\psi_0)] \times [(1+k^2\sigma_1^2)\langle |F'_N|^2 \rangle]^{-1}. \quad (33)$$

Following the arguments used for deriving (27) from (25) we can rewrite (33) as

$$z(\mathbf{H}) = (C_4/\varepsilon p)E_P^2 + C_5 y_Q^2 + [C_6/(\varepsilon p)^{1/2}]E_P y_Q \times (\cos 2\pi\psi_0 + k \sin 2\pi\psi_0), \quad (34)$$

where

$$C_4 = (1+k^2)\sigma_1^2/(1+k^2\sigma_1^2),$$
$$C_5 = \sigma_2^2/(1+k^2\sigma_1^2),$$
$$C_6 = 2\sigma_1\sigma_2/(1+k^2\sigma_1^2). \quad (35)$$

From (34) we can readily obtain $z(\bar{\mathbf{H}})$ to be

$$z(\bar{\mathbf{H}}) = (C_4/\varepsilon p)E_P^2 + C_5 y_Q^2 + [C_6/(\varepsilon p)^{1/2}]E_P y_Q \times (\cos 2\pi\psi_0 - k \sin 2\pi\psi_0) \quad (36)$$

where we have used (18).

In the rest of this section we shall derive the theoretical expression for $M(X_0, y_t)$ for the space-group category 5. The results for the other three categories can be readily obtained by following similar arguments.

For category 5 E_P is a function of θ_i, ϕ_i, ψ_i, $i=1$ to p (for a definition of these see Foster & Hargreaves, 1963*a*). θ_i, ϕ_i and ψ_i ($i=1$ to p) are mutually independent random variables uniformly distributed in the interval 0 to 2π. Let θ'_i, ϕ'_i and ψ'_i ($i=1$ to p) be defined by

$$\theta'_i=\theta_i/2\pi,\quad \theta'_i=\phi_i/2\pi,\quad \psi'_i=\psi_i/2\pi. \qquad (37)$$

It follows that the random variables θ'_i, ϕ'_i and ψ'_i, $i=1$ to p, are mutually independent and uniformly distributed in the interval 0 to 1. Since E_P is a function of θ_i, ϕ_i and ψ_i ($i=1$ to p) it follows that E_P is also a function of θ'_i, ϕ'_i and ψ'_i ($i=1$ to p) [see (37)].

That is,

$$E_P=E_P(\theta'_i,\phi'_i,\psi'_i,\ldots,\theta'_p,\phi'_p,\psi'_p). \qquad (38)$$

The joint probability density function of the random variables* y_Q, ψ'_0, θ'_1, ϕ'_1, $\psi'_1,\ldots,\theta'_p$, ϕ'_p, ψ'_p is known to be (Parthasarathy & Ponnuswamy, 1981*b*)

$$\begin{gathered}P(y_Q,\ldots,\psi'_p)=2y_Q\exp(-y_Q^2),\\ 0\le y_Q<\infty,\quad 0\le\psi'_0,\theta'_1,\ldots,\psi'_p<1.\end{gathered} \qquad (39)$$

From (27), (34) and (36) [see also (38)] it is clear that

$$X=X(y_Q,\ldots,\psi'_p) \qquad (40)$$

and [see (10)]

$$y_{\min}=y_{\min}(y_Q,\ldots,\psi'_p). \qquad (41)$$

In the $(3p+2)$-dimensional $(y_Q,\ldots,\psi'_p)$ space let the domain satisfying the conditions $X\ge X_0$ and $y_{\min}\ge y_t$ simultaneously be denoted by D. In view of (40) and (41), we obtain from (39)

$$\begin{aligned}&\Pr[(X\ge X_0)\cap(y_{\min}\ge y_t)]\\ &\quad=\int_D\!\!\ldots\!\int 2y_Q\exp(-y_Q^2)\,\mathrm{d}y_Q\ldots\mathrm{d}\psi'_p,\end{aligned} \qquad (42)$$

where the $(3p+2)$-fold integration is over the region D of the $(y_Q,\ldots,\psi'_p)$ space. The expression on the right hand side of (42) is too difficult to evaluate in closed form but may be evaluated by the Monte Carlo method of integration in a digital computer.

It may be noted that E_P for category 6 is also a function of the $3p$ variables $(\theta'_1,\ldots,\psi'_p)$. For category 3, E_P is a function of the $2p$ variables $(\theta'_1,\phi'_1,\ldots,\theta'_p,\phi'_p)$. For category 1, E_P is a function of the p variables $(\theta'_1,\theta'_2,\ldots,\theta'_p)$. The explicit expressions of E_P for the categories 1, 3, 5 and 6 are available in Table 1 of Parthasarathy & Ponnuswamy (1976). Expression (42) is valid for all the categories. It is, however, necessary to note that the multiple integral involves $(p+2)$, $(2p+2)$, $(3p+2)$ and $(3p+2)$ variables for the space-group categories 1, 3, 5 and 6, respectively.

4.2. *Results for the many-atom cases in space group P*1

In this subsection we shall take the space group of the crystal to be $P1$. Here it is convenient to make use of the variables y_Q, y_P and ψ instead of $(y_Q,\ldots,\psi'_p)$. Since y_Q, y_P and ψ are independent random variables (Parthasarathy & Srinivasan, 1964), their joint density function will be the product of their marginal densities. That is,

$$P(y_Q,y_P,\psi)=P(y_Q)P(y_P)P(\psi). \qquad (43)$$

$P(y_Q)$ is given by the acentric Wilson distribution and ψ is uniformly distributed in the interval 0 to 2π (Parthasarathy & Srinivasan, 1964). $P(y_P)$ for the cases $P=MN$ and $P=MC$ are the acentric and centric Wilson distributions respectively (Wilson, 1949). We thus have

$$\begin{aligned}P(y_Q,y_P,\psi)&=\frac{2}{\pi}y_Py_Q\exp(-y_Q^2-y_P^2)\qquad\text{for } P=MN\\ &=\frac{2^{1/2}}{\pi^{3/2}}y_Q\exp(-y_Q^2-y_P^2/2)\\ &\qquad\qquad\qquad\qquad\text{for } P=MC,\\ &0\le y_Q,\quad y_P<\infty,\quad 0\le\psi\le2\pi.\end{aligned} \qquad (44)$$

Equation (26) is valid for the present cases. From (33) we can readily show that [see also (16)]

$$z(\mathbf{H})=C_4y_P^2+C_5y_Q^2+C_6y_Py_Q(\cos\psi+k\sin\psi). \qquad (45)$$

From (45) we can readily obtain [see (18)]

$$z(\bar{\mathbf{H}})=C_4y_P^2+C_5y_Q^2+C_6y_Py_Q(\cos\psi-k\sin\psi). \qquad (46)$$

From (26) it is seen that X is a function of y_Q, y_P and ψ. From (45) and (46) and the definition of $y_{\min}$ it is seen that

$$y_{\min}=y_{\min}(y_P,y_Q,\psi). \qquad (47)$$

By following the arguments used for deriving (42) we can show that for the present cases

$$\begin{aligned}&\Pr[(X\ge X_0)\cap(y_{\min}\ge y_t)]\\ &\quad=\iiint_D P(y_Q,y_P,\psi)\,\mathrm{d}y_Q\,\mathrm{d}y_P\,\mathrm{d}\psi,\end{aligned} \qquad (48)$$

where D is now the domain in the three-dimensional (y_Q,y_P,ψ) space satisfying the conditions $X\ge X_0$ and $y_{\min}\ge y_t$ simultaneously. The theoretical expressions for the cases $P=MN$ and $P=MC$ are to be obtained by substituting the appropriate expression for $P(y_Q,y_P,\psi)$ from (44) in (48). These integrals are too complicated to be evaluated in closed form and were therefore evaluated by the Monte Carlo method.

5. Discussion of the theoretical results

$M(X_0,y_t)$, for given X_0 and y_t, is a function of k and σ_1^2. In actual crystals the truncation limit y_t is in the neighbourhood of 0·2 (Ponnuswamy & Parthasarathy, 1977). The BD data of reflections for

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* For brevity we shall denote $(y_Q,\psi'_0,\theta'_1,\phi'_1,\psi'_1,\ldots,\theta'_p,\phi'_p,\psi'_p)$ by $(y_Q,\ldots,\psi'_p)$.

Table 1. *Values of* $M(0{\cdot}1, 0{\cdot}3)$ *(in %) as a function of k and* σ_1^2 *for crystals belonging to the space group categories* 1, 3, 5 *and* 6: $p = 1$

							$\sigma_1^2 \rightarrow$						
k	*	5	10	15	20	25	30	40	50	60	70	80	90
0·04	1	0	4	9	12	15	18	21	21	19	16	11	3
	3	1	5	8	10	12	14	15	16	14	12	8	3
	5	2	5	8	10	12	13	15	15	15	13	10	4
	6	2	5	7	9	10	10	12	12	11	9	7	3
0·06	1	6	15	22	28	32	34	36	36	34	30	22	10
	3	7	13	18	22	24	26	27	27	26	26	18	10
	5	7	13	18	21	24	26	28	28	27	24	20	12
	6	6	12	15	18	20	22	22	22	20	18	15	9
0.08	1	13	26	35	40	43	45	46	46	43	40	34	19
	3	13	22	28	32	34	35	37	37	36	33	28	17
	5	12	21	27	31	34	36	38	39	37	35	30	19
	6	12	19	23	26	28	30	31	31	30	27	23	14
0·10	1	22	36	45	49	52	53	53	53	51	49	42	38
	3	19	30	35	35	39	41	43	44	45	43	41	36
	5	18	28	36	39	42	44	46	46	45	43	38	27
	6	17	25	29	32	34	36	38	38	37	35	30	21
0·12	1	30	45	52	55	57	58	58	58	57	55	49	35
	3	25	36	41	45	47	48	50	51	49	47	42	31
	5	24	36	42	46	48	50	52	52	51	49	45	34
	6	22	30	34	38	40	42	44	44	43	41	37	27
0·14	1	37	52	57	60	61	62	62	62	61	60	55	42
	3	30	41	46	49	51	52	54	54	54	51	47	36
	5	29	41	47	51	53	54	56	56	56	54	50	39
	6	26	34	39	42	44	45	48	48	47	45	41	31
0·16	1	43	57	61	63	64	64	65	64	64	63	59	48
	3	35	45	50	52	54	55	57	58	57	55	51	41
	5	34	47	52	55	57	58	59	60	59	57	54	44
	6	30	38	43	46	48	49	51	52	51	59	45	36
0·18	1	49	60	64	65	66	67	67	67	66	66	63	52
	3	39	49	53	55	57	58	60	60	60	58	54	44
	5	39	50	56	58	60	61	62	62	62	60	57	48
	6	33	41	46	49	50	52	54	55	53	52	48	39
0·20	1	53	63	66	67	68	68	69	69	68	68	66	56
	3	43	51	55	58	59	60	61	62	62	61	57	47
	5	43	53	58	61	62	63	64	64	64	62	60	51
	6	36	44	48	51	53	54	56	56	55	54	51	42
0·22	1	57	65	68	69	69	70	70	70	70	70	68	59
	3	45	54	57	59	60	62	63	64	64	63	60	50
	5	46	56	60	63	64	65	66	66	66	64	62	54
	6	39	46	50	53	55	56	58	58	58	56	53	45
0·24	1	60	67	69	70	70	71	71	71	71	72	70	62
	3	48	55	59	61	62	63	65	65	65	64	61	52
	5	49	58	62	64	66	67	68	68	67	66	64	56
	6	41	48	52	54	56	58	59	59	59	58	54	47
0·26	1	62	68	70	71	71	72	72	72	73	73	72	65
	3	50	57	61	62	63	64	66	66	66	66	63	54
	5	52	60	64	66	67	68	69	69	68	67	65	58
	6	43	50	53	56	58	59	60	60	61	59	56	49
0·28	1	64	69	71	72	72	72	73	73	74	74	74	67
	3	52	59	62	63	65	66	67	67	67	67	64	56
	5	54	62	65	67	68	69	70	70	69	68	67	60
	6	44	51	55	57	59	60	61	62	62	60	57	50
0·30	1	65	70	72	73	73	73	73	74	74	75	75	68
	3	54	60	63	64	66	67	68	68	69	68	65	58
	5	56	63	67	68	69	70	70	70	70	69	68	61
	6	46	53	56	58	60	61	62	63	63	61	59	52
0·35	1	68	72	74	74	74	74	74	75	76	78	78	72
	3	57	62	65	67	68	69	70	70	71	70	68	61
	5	60	66	69	70	71	71	72	72	72	71	69	65
	6	49	55	58	61	62	63	64	65	64	63	61	54
0·40	1	71	74	75	75	75	75	75	76	78	79	80	74
	3	60	64	67	69	69	70	71	72	72	72	69	63
	5	63	68	70	71	72	73	73	73	72	72	71	67
	6	52	57	60	62	63	64	65	66	65	65	62	56
0·45	1	72	75	75	75	75	75	76	77	79	81	81	76
	3	62	66	68	70	70	71	72	73	74	73	71	64
	5	65	69	71	72	73	74	74	74	73	73	72	68
	6	54	59	62	64	64	65	66	66	67	66	64	58
0·50	1	73	75	76	75	75	76	77	78	80	82	83	78
	3	64	67	69	71	71	72	73	74	75	74	72	65
	5	66	70	72	73	74	74	74	74	74	73	73	69
	6	56	61	63	64	66	67	67	67	68	66	64	58
0·55	1	74	76	76	76	76	76	77	79	81	83	83	79
	3	65	68	70	72	72	73	74	75	75	74	72	66
	5	68	71	73	74	74	75	75	75	74	74	73	70
	6	57	62	64	66	67	67	67	68	68	67	65	59
0·60	1	74	76	76	76	76	76	78	80	82	84	84	80
	3	66	69	71	72	73	74	75	75	76	75	73	67
	5	69	72	73	74	75	75	75	75	75	74	73	71
	6	58	63	64	66	67	68	68	68	69	68	65	59

Note. σ_1^2 is in %.

* Numbers in this column denote the space-group category.

Table 2. *Values of* $M(0{\cdot}1, 0{\cdot}3)$ *(in %) as a function of k and* σ_1^2 *for crystals belonging to the space group categories* 1, 3, 5 *and* 6: $p=2$

$\sigma_1^2 \rightarrow$

k	*	5	10	15	20	25	30	40	50	60	70	80	90
0·04	1	1	5	8	10	12	13	15	15	14	11	8	3
	3	2	4	7	10	12	13	15	15	14	12	10	5
	5	1	5	7	10	11	13	14	14	14	13	9	5
	6	2	5	7	9	11	12	14	14	13	11	9	4
0·06	1	6	13	18	22	24	25	27	26	25	23	17	9
	3	6	12	17	21	24	26	27	28	27	24	20	12
	5	6	13	17	21	23	25	27	28	27	24	20	12
	6	6	12	17	20	22	24	26	26	25	23	19	11
0·08	1	13	22	28	31	34	35	36	36	35	33	27	17
	3	12	21	27	31	34	35	37	38	37	34	30	20
	5	12	21	27	31	34	37	38	38	37	35	30	20
	6	12	21	26	29	32	34	35	36	36	33	28	18
0·10	1	19	30	35	38	40	42	43	44	43	40	35	24
	3	18	28	35	39	42	44	46	46	45	42	38	27
	5	18	28	36	40	42	44	46	46	44	43	37	27
	6	18	27	33	37	39	41	42	44	42	41	36	26
0·12	1	25	36	40	44	46	47	49	49	49	47	42	30
	3	24	35	42	46	48	50	52	52	51	48	45	34
	5	24	35	42	46	48	49	51	52	51	49	44	33
	6	23	34	39	43	44	46	48	49	49	47	43	33
0·14	1	31	40	45	48	50	51	53	53	53	51	46	36
	3	29	41	47	50	52	54	56	57	56	53	49	40
	5	29	41	47	50	52	54	56	56	55	53	49	39
	6	28	39	44	47	49	50	52	53	53	52	48	37
0·16	1	35	44	49	49	53	54	56	56	57	55	51	40
	3	34	45	51	54	56	58	59	60	59	57	53	44
	5	34	46	51	54	56	57	58	59	58	57	53	44
	6	32	43	48	50	52	53	55	57	57	55	52	42
0·18	1	39	47	52	54	56	57	58	59	59	58	54	44
	3	38	49	54	57	59	61	62	62	62	60	57	47
	5	38	49	54	57	59	60	61	61	61	59	56	47
	6	37	46	51	53	56	57	58	60	59	58	55	45
0·20	1	42	50	54	57	58	59	61	61	62	60	57	47
	3	42	52	57	60	61	62	64	64	63	62	59	51
	5	42	52	57	59	61	62	64	64	63	62	59	50
	6	40	49	54	56	58	59	61	62	62	60	57	48
0·22	1	44	52	56	58	60	61	62	63	63	62	59	50
	3	45	55	59	62	63	65	66	65	65	64	62	53
	5	45	55	59	62	63	64	65	65	65	64	61	53
	6	43	52	56	59	60	61	63	64	63	61	59	51
0·24	1	46	54	58	60	61	62	64	65	64	64	61	53
	3	48	57	61	64	65	66	67	67	67	65	63	56
	5	49	57	61	63	65	65	66	67	66	65	62	55
	6	45	54	58	61	62	63	64	65	64	63	60	53
0·26	1	49	56	59	61	62	64	65	66	66	65	63	54
	3	50	59	63	66	67	67	68	68	68	67	65	58
	5	51	58	63	65	66	67	68	68	68	66	64	57
	6	48	56	60	62	63	65	66	66	66	64	62	54
0·28	1	51	58	61	62	64	65	67	67	67	66	64	56
	3	52	61	65	67	68	68	69	69	69	68	66	59
	5	53	60	64	66	67	68	69	69	69	68	65	60
	6	50	58	62	63	65	66	67	68	67	66	63	56
0·30	1	52	59	62	63	65	66	68	68	68	68	65	57
	3	54	63	67	68	69	69	70	70	70	69	67	61
	5	54	62	66	67	69	69	70	70	70	69	67	61
	6	52	59	63	65	66	67	68	68	68	67	64	58
0·35	1	56	61	64	66	67	68	70	70	70	70	67	60
	3	58	66	69	70	71	71	72	72	71	71	69	63
	5	58	65	68	70	71	72	72	72	72	71	69	63
	6	55	63	66	67	69	69	70	71	70	69	67	61
0·40	1	58	64	66	67	69	70	71	72	72	72	69	63
	3	62	69	70	71	72	72	73	73	73	72	71	66
	5	61	68	70	71	73	73	73	74	73	73	71	65
	6	58	65	68	69	70	71	71	72	72	70	68	63
0·45	1	61	65	67	69	70	71	72	73	73	73	71	64
	3	65	70	72	72	73	73	73	74	73	73	72	67
	5	64	69	71	73	74	74	74	75	74	74	72	66
	6	61	67	69	70	72	72	72	73	73	71	69	65
0·50	1	62	67	69	70	71	72	73	74	74	74	72	65
	3	67	71	73	73	74	74	74	74	74	74	73	68
	5	66	70	72	74	74	75	75	75	75	74	73	67
	6	63	69	71	72	72	73	73	73	73	72	70	66
0·55	1	64	68	70	71	71	72	74	75	75	74	73	66
	3	68	72	73	74	74	75	74	74	75	75	74	69
	5	68	71	74	75	75	75	76	76	75	75	73	69
	6	65	70	71	72	73	74	74	74	74	72	71	66
0·60	1	65	69	70	71	72	73	75	75	75	75	73	67
	3	70	73	74	75	75	75	75	75	75	75	74	70
	5	69	72	74	75	75	76	77	77	76	75	74	69
	6	67	71	72	73	74	74	74	74	74	73	71	67

Note. σ_1^2 is in %.

* Numbers in this column denote the space-group category.

Table 3. *Values of $M(0\cdot1, 0\cdot3)$ (in %) as a function of k and σ_1^2 for many-atom $P = MN$ and MC cases*

						$\sigma_1^2\rightarrow$						
k		5	10	15	20	25	30	40	50	60	70	80	90
0·04	*MN*	1	4	7	9	11	13	15	15	15	13	10	5
	MC	2	4	6	8	9	10	11	12	12	10	7	4
0·06	*MN*	5	12	17	21	23	25	27	28	28	26	21	12
	MC	5	10	14	17	18	20	21	22	22	21	17	11
0·08	*MN*	11	21	27	30	33	35	38	38	38	36	31	21
	MC	10	17	22	24	27	28	30	31	31	29	26	18
0·10	*MN*	17	29	35	39	42	43	45	46	45	44	39	38
	MC	15	24	28	32	34	35	37	38	38	37	33	24
0·12	*MN*	24	35	41	45	48	49	51	52	51	49	45	34
	MC	20	29	34	37	39	41	43	44	44	42	39	30
0·14	*MN*	29	41	47	50	53	53	56	56	56	54	50	40
	MC	25	33	39	41	44	45	47	48	49	47	43	35
0·16	*MN*	34	45	51	54	56	57	59	60	60	58	54	45
	MC	28	37	42	45	47	48	51	52	53	51	48	39
0·18	*MN*	38	50	54	57	59	60	62	63	63	61	57	49
	MC	32	41	45	48	50	51	54	55	56	54	51	42
0·20	*MN*	42	53	57	60	62	63	64	65	65	63	60	52
	MC	34	44	48	51	53	54	56	57	58	56	53	45
0·22	*MN*	45	55	60	62	64	64	66	67	67	65	62	54
	MC	37	46	50	53	55	56	58	59	60	58	55	47
0·24	*MN*	48	58	61	64	66	66	68	68	68	66	66	57
	MC	40	48	52	55	57	57	60	61	61	60	57	59
0·26	*MN*	51	60	63	66	67	67	69	69	69	68	65	58
	MC	42	50	54	57	58	59	61	62	63	61	58	51
0·28	*MN*	53	61	65	67	68	69	70	71	70	69	66	60
	MC	44	52	56	58	59	61	63	64	64	62	60	52
0·30	*MN*	55	63	66	68	69	70	71	71	71	69	67	61
	MC	46	53	57	59	61	62	64	65	65	63	60	53
0·35	*MN*	59	66	68	70	71	71	72	72	72	71	69	64
	MC	49	57	60	62	63	64	66	67	66	65	62	56
0·40	*MN*	62	68	70	72	72	73	73	73	73	72	71	66
	MC	52	59	62	64	65	66	67	68	67	66	64	58
0·45	*MN*	65	70	72	73	73	74	74	74	74	73	72	68
	MC	55	61	64	66	67	68	68	69	69	67	66	60
0·50	*MN*	67	71	73	74	74	74	74	74	74	74	72	69
	MC	57	63	66	67	68	69	69	69	69	68	65	60
0·55	*MN*	68	73	74	74	75	74	74	75	75	74	73	70
	MC	59	64	67	68	69	69	69	70	70	68	66	60
0·60	*MN*	70	73	75	75	75	75	75	75	75	74	73	70
	MC	60	66	68	69	70	70	70	70	70	68	66	61

Note. σ_1^2 is in %.

which $y(\mathbf{H})$ and $y(\bar{\mathbf{H}})$ are close to this limit may not be very accurate. We shall therefore assume that reflections for which $y(\mathbf{H})$ and $y(\bar{\mathbf{H}})$ are greater than 0·3 would be sufficiently accurate to yield useful BD data. A BR whose value is about 0·1 or more could be measured fairly accurately. We shall therefore take $M(0\cdot1, 0\cdot3)$ as an index of the measurability of BDs of a crystal. Values of $M(0\cdot1, 0\cdot3)$ have been calculated for triclinic, monoclinic and orthorhombic crystals containing p (= 1 or 2) anomalous scatterers per asymmetric unit for different fixed values of k and σ_1^2 by evaluating the appropriate integrals of § 4 by the Monte Carlo method (see Demidovich & Maron, 1973). The results obtained are given in Tables 1 and 2 for $p = 1$ and 2, respectively. The results obtained for the many-atom cases (*i.e.* $P = MN$ and MC) of space group $P1$ are given in Table 3.

5.1. *Comparison with earlier results*

In the earlier study the measurability values were obtained (for crystals of space group $P1$) from the probability distribution of δ instead of from that of X (Parthasarathy & Ponnuswamy, 1981*a*). This was done in order to avoid theoretical complications as well as to obtain the theoretical expressions for the measurability corresponding to the various cases in a simple form. It is relevant to compare the theoretical values of the measurability as obtained from the distributions of X (*i.e.* the present theory) and δ.

The expression for δ is obtained from that of X by neglecting the positive quantity $|F''_P|^2$ in the denominator of the expression for X [see (6) and (7)]. Therefore it follows that the value of δ for any given reflection will always be greater than that of X. This implies that the theoretical value of the measurability as obtained from the probability distribution of δ would always be an overestimated value compared with the true value that would be obtained from the distribution of X. This overestimation would become more pronounced with increasing value of k (*i.e.* under large AS effect). A comparison of the values of the measurability as obtained from the earlier theory of Parthasarathy & Ponnuswamy (1981*a*) for space group $P1$ with the corresponding values obtained from the present theory confirms these features (see Table 4).

5.2. *Remark on relevant values of σ_1^2*

Since light-atom structures containing less than 100 atoms* per asymmetric unit can in principle be determined by direct methods, the AS method may not be preferred for determining such structures from their heavy-atom derivatives. In our discussion we shall therefore assume the structures to be sufficiently complex (say $n > 100$) and for such structures the AS method would be more suited than any other single method. For complex structures the values of σ_1^2 would not generally be large ($\sigma_1^2 < 0\cdot5$ say). For the heavy-atom derivatives of proteins σ_1^2 will often be quite small† ($\sigma_1^2 < 0\cdot2$, say). In our discussion regarding the measurability we shall therefore confine ourselves to the region $\sigma_1^2 < 0\cdot5$.

5.3. *Variation of measurability as a function of k*

The variation of M $(0\cdot1, 0\cdot3)$ as a function of k for different fixed values of σ_1^2 is shown in Fig. 2. These curves correspond to the situation where the crystal of space-group category 3 contains one anomalous scatterer (*i.e.* $p = 1$) per asymmetric unit besides the other light atoms. For a given σ_1^2, as k increases, the measurability increases almost linearly and somewhat steeply until k is about 0·15 and the rate of increase becomes less for values of k up to 0·25 (see Fig. 2). For larger values of k the curves become somewhat linear with a very small slope. Thus the measurability

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* In our discussion the term 'atoms' is used to refer to the non-hydrogen atoms only.

† For Sm derivatives of the proteins chicken lysozyme (MW 14 297) and substilisin BPN (MW 27 499) with one Sm atom per protein molecule the average values (corresponding to 2 Å data) of σ_1^2 are 0·1 and 0·05, respectively.

Table 4. *Comparison of the measurability of Bijvoet differences (in %) obtained from earlier (ET) and present (PT) theories for a few typical situations*

		0·1			0·2			0·3			0·4		
k	σ_1^2	$P1$	MN	MC	$P1$	MN	MC	$P1$	MN	MC	$P1$	MN	MC
0·1	*ET*	38	29	24	51	40	32	55	45	37	56	47	39
	PT	36	29	24	49	39	32	53	43	35	53	45	37
0·2	*ET*	66	54	45	71	62	53	73	66	57	73	67	59
	PT	63	53	44	67	60	51	68	63	54	69	64	56
0·3	*ET*	74	65	55	78	71	62	79	74	66	79	75	67
	PT	70	63	53	73	68	59	73	70	62	73	71	64
0·4	*ET*	79	71	62	81	76	68	82	78	71	83	79	73
	PT	74	68	59	75	72	64	75	73	66	75	73	67
0·5	*ET*	81	75	67	83	79	72	84	81	75	85	81	76
	PT	75	71	63	75	74	67	76	74	69	77	74	69
0·6	*ET*	83	78	70	85	81	75	85	83	77	86	83	78
	PT	76	73	66	76	75	69	76	75	70	78	75	70

Note. The values against *ET* were obtained from the probability distribution of δ. Those against *PT* are the results of the present theory based on the distribution of X. Note that the values against *ET* are overestimated compared to their corresponding values shown against *PT*.

is a non-linear function of k as expected from the expression for X [see (21)]. It may, however, be noted here that, in the earlier theory, the measurability turned out to be a linear function of k (for all k) and this unwarranted result arose due to an approximation (for details of the approximation see § 5.1) which is not appropriate under a quite pronounced anomalous scattering effect.

5.4. *Variation of measurability as a function of* σ_1^2

The variation of $M(0{\cdot}1, 0{\cdot}3)$ as a function of σ_1^2 for different fixed values of k is shown in Fig. 3 for the situation considered in § 5.3. For a given moderate or large value of k (*e.g.* $k = 0{\cdot}2$) the measurability increases steeply and almost linearly as σ_1^2 increases in the region 0 to 0·1 (see Fig. 3). For values of σ_1^2 in the region 0·1 to 0·25 the rate of this increase becomes much less. For $\sigma_1^2 > 0{\cdot}25$ the curves become practically flat. Since for the heavy-atom derivatives of proteins the values of σ_1^2 are in general small, the feature that in the region of small values of σ_1^2 the measurability increases steeply with increasing value of σ_1^2 is of special interest in protein crystallography.

5.5. *Influence of space-group symmetry on the measurability*

It is seen from Tables 1 and 2 that among crystals containing one anomalous scatterer per asymmetric unit (*i.e.* $p = 1$), for given k and σ_1^2 (*i.e.* for given type of heavy atom, wavelength and complexity of asymmetric unit), the measurability is a maximum for the triclinic category 1 and least for the orthorhombic category 6. Other conditions being the same, measurability values for categories 3 and 5 are somewhat similar and lie between those for categories 1 and 6. In crystals with two heavy atoms per asymmetric unit (*i.e.* $p = 2$; see Table 2) this feature is present to a lesser extent only.

It may incidentally be noted that, for given k and σ_1^2, the measurability is larger for the many-atom case $P = MN$ than for the many-atom case $P = MC$ (see Table 3).

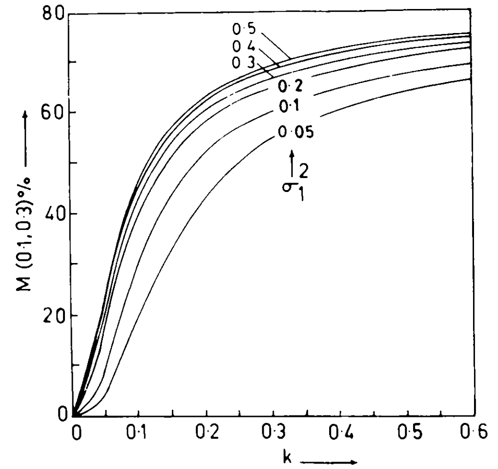

Fig. 2. Variation of the measurability $M(0{\cdot}1, 0{\cdot}3)$ (in %) as a function of k for different fixed values of σ_1^2 for $p = 1$ in category 3.

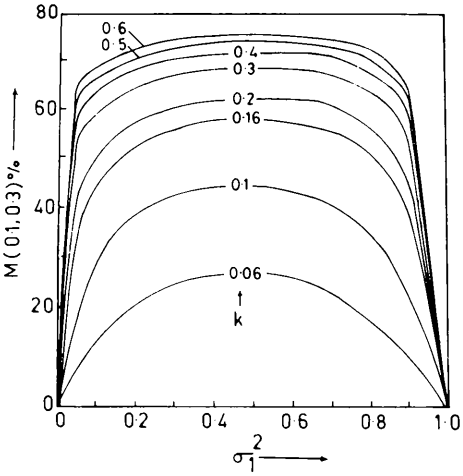

Fig. 3. Variation of the measurability $M(0{\cdot}1, 0{\cdot}3)$ (in %) as a function of σ_1^2 for different fixed values of k for $p = 1$ in category 3.

Table 5. *Measurability of Bijvoet differences (in %) to be expected with* Cu *Kα and with a radiation of* λ = 1·8448 Å *in macromolecular crystals of space group categories* 1, 3, 5 *and* 6 *containing* p (= 1 *or* 2) Sm *atoms and* q (= 500, 1000, . . . , 3000) *normal scatterers per asymmetric unit*

		Results for Cu *Kα*					Results for λ = 1.8448 Å				
			Space-group category					Space-group category			
q	p	σ_1^2	1	3	5	6	σ_1^2	1	3	5	6
500	1	20·3	73	64	67	56	12·1	77	73	76	69
	2	33·7	65	70	67	63	21·5	76	79	75	72
1000	1	11·3	70	60	63	52	6·4	77	71	74	65
	2	20·3	63	67	66	64	12·1	75	77	76	73
1500	1	7·9	68	57	59	48	4·4	77	69	73	62
	2	14·6	61	64	64	61	8·4	74	75	75	72
2000	1	6·0	66	53	56	45	3·3	77	68	71	61
	2	11·3	60	62	62	58	6·4	72	74	74	71
2500	1	4·9	64	52	54	43	2·7	76	67	71	60
	2	9·3	57	59	60	55	5·2	71	73	73	70
3000	1	4·1	61	49	51	41	2·2	76	66	69	59
	2	7·9	56	58	59	54	4·4	69	72	73	69

For Sm, $\langle k \rangle$ = 0·270 and 0·859 for 2 Å data with Cu *Kα* and for the radiation with λ = 1·8448 Å, respectively. σ_1^2 values are in %.

5.6. *Measurability in* Sm *derivatives of proteins with* Cu *Kα radiation*

Since Sm exhibits a large anomalous scattering effect for Cu *Kα*, in this section we shall discuss the measurability of BDs to be expected in Sm derivatives of proteins of different complexity. We shall consider situations where the following typical conditions are satisfied: (i) the number of normal scatterers per asymmetric unit is a few thousands (*i.e.* q = 500, 1000, 1500, . . . , 3000); (ii) the composition of the q atoms is such that 80% of these are carbons, 10% are nitrogens and 10% are oxygens; (iii) the crystals are such that data corresponding to 2 Å resolution can be collected. The values of $M(0{\cdot}1, 0{\cdot}3)$ for a few typical macromolecular situations are given in Table 5. The average values of σ_1^2 and k (for Cu *Kα*) corresponding to 2 Å resolution data are also given. From this table it is seen that a measurability of more than 40% could be achieved in macromolecular crystals containing even a few thousand atoms per asymmetric unit by a judicious choice of the wavelength, type of heavy atom and their number (*i.e.* one or more) per asymmetric unit.

5.7. *Comparison of measurabilities in* Sm *derivatives of a protein for* Cu *Kα and for radiation with* λ = 1·8448 Å

Recently, Templeton, Templeton, Phizackerley & Hodgson (1982) have experimentally measured the values of f' and f'' for Sm at various wavelengths and found f'' to be the largest at λ = 1·8448 Å. For Sm the average values of k (for 2 Å resolution data) are 0·27 and 0·859 for Cu *Kα* and for λ = 1·8448 Å, respectively. Thus the value of k of Sm for λ = 1·8448 Å is *more than three times* its value for Cu *Kα*. It would hence be interesting to compare the values of the measurability for these two radiations in a few typical cases of Sm derivatives of molecules containing a few thousand atoms in the asymmetric unit. The relevant results are given in Table 5. It is seen that for a derivative with one Sm and 2000 other atoms per molecule crystallizing in a space group of category 3 the measurability values are 53 and 68% for Cu *Kα* and for λ = 1·8448 Å, respectively. Thus, in spite of the threefold increase in the value of k for λ = 1·8448 Å (relative to the value of k for Cu *Kα*) the measurability has increased only by 28%. Similar results are obtained for other values of p and in other space group categories (see Table 3). The reasons for the lack of spectacular improvement in the measurability for λ = 1·8448 Å are the following: (i) the non-linear functional dependence of the measurability on k (see § 5.3) and (ii) the reduction in the value of σ_1^2 by nearly 50% relative to its value for Cu *Kα*.

Since the measurability is a non-linear function of k with decreasing slope and since an increase in the value of k is simultaneously accompanied by a decrease in the value of σ_1^2 as one approaches an absorption edge of a heavy atom owing to physical reasons and since in the region of small σ_1^2 the measurability is sensitive with respect to changes in σ_1^2 (see § 5.4) it appears that we may not be able to obtain spectacular increase in measurability by approaching the absorption edge very closely.* However, in macromolecules containing more than a few thousand atoms, in order to tackle the phase problem as effectively as possible, it may become necessary to increase the measurability as much as one can and in order to do this it may then be advantageous to use a radiation whose wavelength is closer to the absorption edge of the heavy atom than Cu *Kα*.

6. Test of theoretical results

The theoretical results obtained in this paper have been tested in the case of a few hypothetical three-dimensional structures. The details of the structures chosen are given in Table 6. Structure (1) was obtained from that of L-*N*-acetylhistidine monohydrate (Kistenmacher, Hunt & Marsh, 1972) by taking one of the atoms in the unit cell to be chlorine and the remaining atoms to be carbons. Structure (2) was obtained by replacing Cl of structure (1) with a cobalt atom. Structure (3) was derived from that of *tert*-butyloxycarbonyl-L-alanyl-L-proline monohydrate

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* (1) As one approaches an absorption edge of an atom f' decreases (*i.e.* $|f'|$ increases) so that the total real part $f^0 + f'$ decreases. This causes σ_1^2 to decrease [see (15)]. This reduction in σ_1^2 is inevitable since it arises due to physical reasons. (2) In the region of very small values of σ_1^2 (*i.e.* $\sigma_1^2 < 0{\cdot}1$) the variation in σ_1^2 affects measurability quite significantly (§§ 5.2 and 5.4).

(Ponnuswamy, 1979) by taking one of the atoms in the asymmetric unit to be a Cl and the remaining atoms to be carbons. Structure (4) was derived from that of (3) by replacing the Cl with a Co atom.

In these structures each atom was assumed to have the same isotropic temperature factor, namely $B = 2{\cdot}5\ \text{Å}^2$. Values of X, k and σ_1^2 were computed for each independent noncentrosymmetric reflection for the data in the range $1/a_{\min} \le \sin\theta/\lambda \le 0{\cdot}6\ \text{Å}^{-1}$, where $a_{\min}$ is the minimum cell dimension of the crystal (Wilson, 1949). The mean values of k and σ_1^2 obtained for the various structures are given in Table 6. The variation of $M(X, 0{\cdot}3)$ as a function of the Bijvoet ratio X has been computed for each structure from the calculated values of X for the various reflections. This variation is shown in Fig. 4 for the four structures by crosses (which are taken to represent the experimental points). The theoretical curve of $M(X, 0{\cdot}3)$ *vs* X was obtained in each case by making use of the appropriate average values $\langle k\rangle$ and $\langle\sigma_1^2\rangle$ for the respective data. The theoretical curves are shown by solid lines. It is seen that there is good agreement between theory and experiment in all the cases.

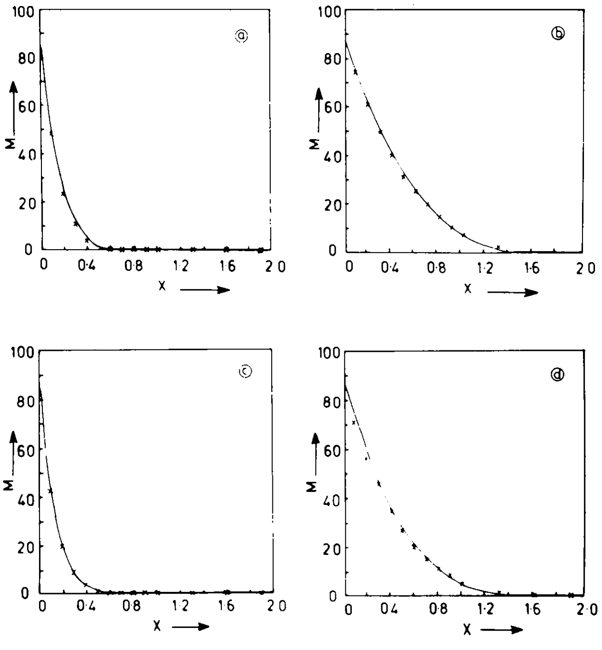

Fig. 4. Verification of theoretical results: variation of $M(X_0, 0{\cdot}3)$ (denoted by M) as a function of X_0 for the structures 1 to 4 of Table 6. In each case the solid line denotes the theoretical curve and crosses denote the experimental data.

Table 6. *Details of the structure taken for testing the theoretical results*

No.	Space group	Space-group category number	p	Asymmetric unit	$\langle k\rangle$	$\langle\sigma_1^2\rangle$
1	$P1$	1	1	ClC_{29}	0·087	0·396
2	$P1$	1	1	CoC_{29}	0·344	0·533
3	$P2_12_12_1$	5	1	ClC_{20}	0·088	0·491
4	$P2_12_12_1$	5	1	CoC_{20}	0·349	0·623

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