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A Semi-empirical Absorption-Correction Technique for Symmetric Crystals in Single-Crystal X-ray Crystallography. I

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A new semi-empirical absorption-correction procedure is described, applicable to X-ray diffraction data collected on a four-circle diffractometer from a single crystal with at least one crystallographic twofold rotation symmetry. The technique is basically a modification of those procedures that make use of ψ-scan intensity variations and is primarily intended for biological macromolecular samples, although it is not limited to such samples. This is the first part of a two-part series and deals with the case wherein the symmetry axis is along the instrument φ axis and the data are collected in the bisecting mode.

Introduction

In order to determine absorption-correction factors empirically, intensities of one or more reflections must be measured at many different crystal orientations. This can be done with symmetry equivalents when the crystal is highly symmetric and/or by means of ψ scans (rotation about the diffraction vector). When the data are collected on a four-circle diffractometer, one class of techniques is to represent the transmission factor as a polynomial function of the various setting angles of the diffractometer and fit it to the intensities of many, more or less randomly chosen, reflections at many ψ angles and symmetry-equivalent positions (Kopfmann & Huber, 1968; Katayama, Sakabe & Sakabe, 1972; Flack, 1974). These techniques require many ψ scans and/or a high degree of crystal symmetry. The purpose of this paper is to show a way of making empirical absorption corrections with a minimum number of ψ scans. The technique requires a twofold rotation symmetry. It is designed particularly for protein crystallography and makes use of approximations that are reasonable for such an application.

The possibility of using a ψ scan for the determination of transmission factors was first suggested by Furnas (1957). If the intensity of a reflection (a reference reflection) is measured as a function of the angle of rotation ψ around its diffraction vector, the observed variation should give relative transmission factors for that reflection as a function of ψ. In this paper we consider the case where the reference reflection is along the instrument φ axis. The ψ rotation is then physically the same as the φ rotation. Furnas's suggestion was to treat the transmission factor for any reflection **h** to be a function of one angle only, the azimuthal angle φ of the diffraction vector of the reflection. Thus, the relative transmission factor of **h** diffracting at $\varphi_{\mathbf{h}}$ was assumed to be the same as that of the reference reflection diffracting at the same φ angle (Fig. 1*a*). Note that in this approximation, the incident and reflected beams of the reflection **h** follow

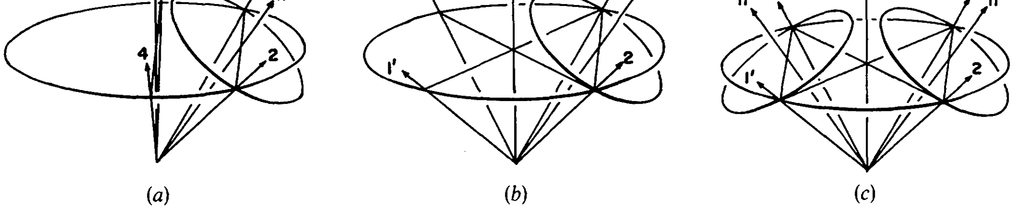

Fig. 1. Directions of the incident and reflected beams of a general reflection **h** and of the reference reflection **k** on the same *k* level. (*a*) Furnas's (1957) method. The ray directions of **h** are labeled 1 and 2 and the ray directions of **k** are labeled 3 and 4. These ray directions are related in pairs by a mirror plane through the vectors **h** and **k**. (*b*) North, Phillips & Mathews's (1968) method. The ray directions of **h** are labeled 1 and 2. The ray directions of **k** are 1 and 1′ at one ψ angle and 2 and 2′ at another. These two ψ angles are chosen strategically so that one of the ray directions of **k** at either ψ angle coincides with a ray direction of **h**. (*c*) Proposed method. The geometry is the same as in (*b*) but it is recognized that the remaining ray directions of **k**, labeled 1′ and 2′, coincide with the ray directions of **h**′ which is related to **h** by the twofold symmetry about **k**.

a path that is entirely different from that of those of the reference reflection **k**.

North, Phillips & Mathews (1968) improved on this technique basically by setting the transmission factor of a reflection **h** equal to the average of those of the reference reflection at two particular angles ψ_1 and ψ_2. These angles are chosen such that, at each of these angles, one of the two ray directions of the reference reflection coincides with a ray direction of **h** (Fig. 1*b*). This procedure results in a significant improvement over those obtainable by Furnas's procedure but still contains a major unsatisfactory feature in that the absorption effect of the remaining two rays of the reference reflection at these ψ angles becomes improperly associated with that of the reflection **h**. It will be shown in this paper that these remaining ray directions in fact coincide with the incident and reflected beams of a reflection **h**′, which is related to **h** by the crystallographic twofold rotation symmetry (Fig. 1*c*) if (*a*) the crystal is mounted such that its crystallographic twofold axis is coincident with the instrument φ axis, (*b*) the ψ scan is made about this axis and (*c*) the intensities of the general reflections **h** and **h**′ are measured in the bisecting mode ($\omega=\theta$). Improvement will, therefore, result if the intensities of both **h** and **h**′ are measured and the transmission factor applicable to the *average* of these two intensities is set equal to the average of the transmission factors of the reference reflection at these ψ angles.

The relative transmission factors estimated in this way are applicable to all reflections in one layer but they must be correlated to those of other layers along the symmetry axis by putting them on a common scale. Determination of these level scale factors is a difficult task and, in protein crystallography, these are often ignored. This practice amounts to assuming that the transmission factor is independent of the polar angle (the diffractometer χ angle if the ψ scan axis coincides with the instrument φ axis). The polar-angle dependency can be obtained by means of additional ψ scans about reflections (the secondary reference reflections) whose diffraction cones intersect with those of the primary reference reflections. It is easy to see that the most economical way, in terms of reducing the number of necessary ψ scans, is to use as the secondary reference those reflections whose diffraction cones are perpendicular to those of the primary references. The experimental feasibility of making such a scan will be examined closely in this paper.

These procedures are formally justifiable only if one considers the transmission factor to be separable into two parts, one for the incident beam and another for the diffracted beam. The underlying assumption, therefore, is that the transmission factor is independent of the scattering angle θ. The θ dependency may be approximately corrected by the use of the spherical transmission factor table (Kopfmann & Huber, 1968; Flack, 1974). To this end it is necessary to determine μR, the product of the effective linear absorption coefficient and the effective radius of the sample. A practical way of determining this value will be suggested and discussed.

Overall procedure

Our procedure is based on the assumption that the transmission factor of a given reflection can be separated into three independent parts.

$$T=R(i)R(r)S(\theta,\mu R)\,. \tag{1}$$

The factor R is assumed to depend only on the orientation of the incident (i) or reflected (r) X-ray beam with respect to the sample. S is the spherical transmission factor at the Bragg scattering angle θ. μR is the effective value of the product of the linear absorption coefficient and the radius of the sphere when the sample is considered to be a uniformly absorbing sphere.

The approximation (1) was proposed by Kopfmann & Huber (1968) who also confirmed its essential validity by a semi-empirical testing. (They did not explicitly give the value of μR but, from the dimensions of the hypothetical crystal and the value of μ that they used, it may be estimated to be about 1.) The idea of separating T into two factors – the spherical transmission factor and another that depends only on the orientation of the crystal with respect to the X-ray beams and which here is assumed to be $R(i)R(r)$ – was also proposed by Flack (1974). The separation of the orientation-dependent part into two factors – one for the incident beam and the other for the reflected beam – has also been proposed by North, Phillips & Mathews (1968), although these authors used the arithmetic mean instead of the product.

R can be considered to be a function of two angles, ϱ and σ. ϱ is the polar angle of the X-ray beam with respect to a reference axis of the crystal and σ is the azimuthal angle. In this and the following (Lee & Ruble, 1977) paper, we consider crystals with at least one twofold rotation symmetry and choose this symmetry axis as our reference axis. For concreteness we shall call this axis **b**. In this paper we consider the case where the crystal is mounted such that this axis is parallel to the instrument φ axis, in which case σ corresponds to the instrument φ and ϱ corresponds to the instrument χ angles. The general case where the crystal is mounted in arbitrary orientation will be discussed in the following paper.

Our procedure, being a modification of that of North, Phillips & Mathews (1968), begins by determining the σ dependence of $R(i)$ and $R(r)$ with the ψ scans of 0*k*0 reflections. For many protein samples this determination must be done frequently during the course of data collection because the position and orientation of the crystal and the mother liquor may shift significantly relative to each other and to the glass capillary during the data collection.

After the σ dependence is determined at several layers of k, the ϱ dependence is determined. This reduces to the determination of level scale factors which in turn is equal to the ratio of the 'true' intensities of different $0k0$ reflections. This is done by means of ψ scans of two reflections $h0l$ and $\bar{h}0\bar{l}$. In most cases, one determination at the end of data collection will suffice.

S can be looked up from the available spherical transmission factor table (Bond, 1967) if μR is known. μR is determined with a pencil of direct beam at the very end of the data collection. Detailed procedures for each of these determinations are described and discussed in the following.

The azimuthal-angle dependence of R

As stated earlier we restrict ourselves in this paper to the case where the crystallographic twofold axis is coincident with the instrument φ axis and the data are collected in the bisecting mode ($\psi=0$, $\omega=\theta$). It is shown in Appendix § 2 that in such a case the incident and reflected ray directions of a general reflection $\mathbf{h}=hkl$ and of its twofold symmetry equivalent $\mathbf{h}'=\bar{h}k\bar{l}$ are coincident with those of the reflection $\mathbf{k}=0k0$ at two particular ψ angles, ψ_1 and ψ_2. (The intensities of a reflection at ψ and $\psi+\pi$ should be identical. When experimentally they are observed to be different, it must mean that the diffractometer alignment is not quite correct, the X-ray beam is not uniform and/or the crystal is not exactly aligned. An average should be taken in such a case. In this paper, we assume that this has been done and that the effective periodicity in ψ is 180°.) Since the ψ scan is done at $\chi=\pm 90°$, ψ and φ are the same angles and we will use φ instead of ψ.

The Kopfmann & Huber (1968) approximation (1) can then be written for $\mathbf{h}$, $\mathbf{h}'$, and $\mathbf{k}$ as follows

$$I_{\mathbf{h}}=R(\mathbf{1})R(\mathbf{2})S(\mathbf{h})I^0_{\mathbf{h}} \tag{2}$$

$$I_{\mathbf{h}'}=R(\mathbf{1}')R(\mathbf{2}')S(\mathbf{h})I^0_{\mathbf{h}} \tag{3}$$

$$I_{\mathbf{k}}(\varphi_1)=R(\mathbf{1})R(\mathbf{1}')S(\mathbf{k})I^0_{\mathbf{k}} \tag{4}$$

$$I_{\mathbf{k}}(\varphi_2)=R(\mathbf{2})R(\mathbf{2}')S(\mathbf{k})I^0_{\mathbf{k}}\,. \tag{5}$$

The superscripts zero in these equations denote the 'true' intensities, *i.e.* the intensity that one would observe if there were no absorption. $\mathbf{1},\mathbf{2},\mathbf{1}'$, and $\mathbf{2}'$ are the various ray directions as shown in Fig. 1(*c*). These equations can then be combined to give

$$I^0_{\mathbf{h}}=\left[\frac{I_{\mathbf{h}}I_{\mathbf{h}'}}{I_{\mathbf{k}}(\varphi_1)I_{\mathbf{k}}(\varphi_2)}\right]^{1/2}\frac{S(\mathbf{k})}{S(\mathbf{h})}I^0_{\mathbf{k}}\,. \tag{6}$$

The two φ angles in (6) can be expressed in terms of the diffractometer setting angles of $\mathbf{h}$ as follows (North, Phillips & Mathews, 1968) (Appendix § 2)

$$\varphi_i=\varphi_{\mathbf{h}}\pm\varepsilon\ (\text{modulo}\ \pi) \tag{7}$$

$$\tan\varepsilon=\tan\theta_{\mathbf{h}}|\cos\chi_{\mathbf{h}}|\,. \tag{8}$$

In these equations $\varphi_{\mathbf{h}}$, $\theta_{\mathbf{h}}$, and $\chi_{\mathbf{h}}$ are the diffractometer setting angles for $\mathbf{h}$ or $\mathbf{h}'$ (either set gives the same result) and ε ranges from 0 to 90°. The upper and lower signs in (7) give φ_1 and φ_2 (modulo 180°).

The polar-angle dependence of R

1. *Principle*

Equation (6) contains $I^0_{\mathbf{k}}$ which is an unknown. If all reflections are confined in the kth layer, this number need not be known since it can be treated as a part of the overall scale factor. However, if the data set spans more than one layer, it is desirable to determine the relative values of these numbers (the level scale factors) experimentally. This can be done by means of ψ scans along directions perpendicular to the symmetry axis.

Suppose that ψ scans were performed for a series of reflections $\mathbf{k}$ along the symmetry axis and that additional ψ scans were performed for a reflection $\mathbf{l}=h0l$ and its twofold symmetry equivalent $\mathbf{l}'=\bar{h}0\bar{l}$. We suppose that the Bragg angle θ for $\mathbf{l}$ and $\mathbf{l}'$ is sufficiently small that their diffraction cones intersect with the diffraction cones of all $\mathbf{k}$.

Referring to Fig. 2, let $\mathbf{1}$ and $\bar{\mathbf{1}}$ be the two ray directions of $\mathbf{l}$ at a particular ψ angle $\psi_{\mathbf{l}}$ such that they lie on the surface of the diffraction cones of the reflection $\mathbf{k}$ at a particular k level and of its Friedel mate $\bar{\mathbf{k}}$ at the level $-k$. Let $\mathbf{1}'$ and $\bar{\mathbf{1}}'$ be the twofold symmetry equivalents of $\mathbf{1}$ and $\bar{\mathbf{1}}$. These will coincide with the ray directions of $\mathbf{l}'$ at a certain ψ angle $\psi_{\mathbf{l}'}$. The important feature of this construction is that the ray directions $\mathbf{1}$ and $\mathbf{1}'$ coincide with the incident and reflected beam directions of the reflection $\mathbf{k}$ at some ψ angle $\psi_{\mathbf{k}}$. Similarly the ray directions $\bar{\mathbf{1}}$ and $\bar{\mathbf{1}}'$ coincide with the incident and reflected beam directions of the reflection $\bar{\mathbf{k}}$ at the ψ angle $\psi_{\bar{\mathbf{k}}}$. The Kopfmann & Huber (1968) approximation (1) can then be written for $\mathbf{k}$, $\bar{\mathbf{k}}$, $\mathbf{l}$, and $\mathbf{l}'$ as follows.

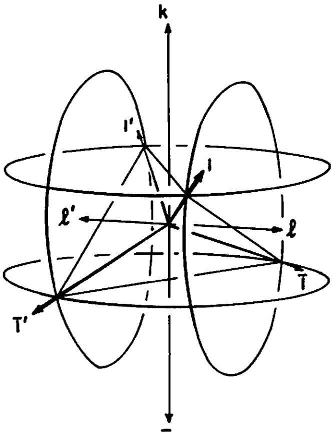

Fig. 2. Intersections of the diffraction cones of the secondary reference $\mathbf{l}$ and its symmetry equivalent $\mathbf{l}'$ with those of the primary reference $\mathbf{k}$ and its Friedel mate $\bar{\mathbf{k}}$. Note that the four ray directions along the intersections of these diffraction cones point toward the corners of a tetrahedron of D_{2d} symmetry.

$$I_{\mathbf{k}}(\psi_{\mathbf{k}}) = R(\mathbf{l})R(\mathbf{l}')S(\mathbf{k})I^0_{\mathbf{k}} \tag{9}$$

$$I_{\bar{\mathbf{k}}}(\psi_{\bar{\mathbf{k}}}) = R(\bar{\mathbf{l}})R(\bar{\mathbf{l}}')S(\mathbf{k})r_{\mathbf{k}}I^0_{\mathbf{k}} \tag{10}$$

$$I_{\mathbf{l}}(\psi_{\mathbf{l}}) = R(\mathbf{l})R(\bar{\mathbf{l}})S(\mathbf{l})I^0_{\mathbf{l}} \tag{11}$$

$$I_{\mathbf{l}'}(\psi_{\mathbf{l}'}) = R(\mathbf{l}')R(\bar{\mathbf{l}}')S(\mathbf{l})I^0_{\mathbf{l}}\,. \tag{12}$$

In (10), $r_{\mathbf{k}}$ is the ratio of the 'true' intensities of the Friedel pair **k** and $\bar{\mathbf{k}}$:

$$r_{\mathbf{k}} = I^0_{\bar{\mathbf{k}}}/I^0_{\mathbf{k}}\,. \tag{13}$$

This number can be set to unity unless the anomalous-scattering effect is unusually large. When the anomalous-scattering effect is very large, this number can still be estimated from the structure determined initially assuming $r=1$.

These equations can be combined to give

$$I^0_{\mathbf{k}} = \left[\frac{1}{r_{\mathbf{k}}}\,\frac{I_{\mathbf{k}}(\psi_{\mathbf{k}})I_{\bar{\mathbf{k}}}(\psi_{\bar{\mathbf{k}}})}{I_{\mathbf{l}}(\psi_{\mathbf{l}})I_{\mathbf{l}'}(\psi_{\mathbf{l}'})}\right]^{1/2}\frac{S(\mathbf{l})}{S(\mathbf{k})}\,I^0_{\mathbf{l}}\,. \tag{14}$$

(14) can be written for each level of k including a particularly chosen reference level k_0. By taking a ratio, the 'true' intensity of **k** at any level can be written relative to that at the reference level by

$$\frac{I^0_{\mathbf{k}}}{I^0_{\mathbf{k}0}} = \left[\frac{r_{\mathbf{k}0}}{r_{\mathbf{k}}}\,\frac{I_{\mathbf{k}}(\psi_{\mathbf{k}})I_{\bar{\mathbf{k}}}(\psi_{\bar{\mathbf{k}}})}{I_{\mathbf{l}}(\psi^k_{\mathbf{l}})I_{\mathbf{l}'}(\psi^k_{\mathbf{l}'})}\,\frac{I_{\mathbf{l}}(\psi^{k_0}_{\mathbf{l}})I_{\mathbf{l}'}(\psi^{k_0}_{\mathbf{l}'})}{I_{\mathbf{k}0}(\psi_{\mathbf{k}0})I_{\bar{\mathbf{k}}0}(\psi_{\bar{\mathbf{k}}0})}\right]^{1/2}\frac{S(\mathbf{k}_0)}{S(\mathbf{k})}\,. \tag{15}$$

2. *Experimental feasibility*

The above procedure requires at least two ψ scans perpendicular to the symmetry axis. When the symmetry axis is parallel to the φ axis, these scans must be done at $\chi=0$. This is not possible to do on conventional four-circle diffractometers because the required ω angle then becomes 90°. This difficulty can be overcome by deliberately changing the goniometer-head arc so that the reflections **l** and **l**′ diffract at $\chi\neq 0$ when $\psi=0$. Since the level scale factor is the ratio of the 'true' intensities of two reflections, it does not depend on the orientation of the crystal. Therefore, the scale factor determined at this new orientation should be applicable to the data collected at the old orientation. The amount of the goniometer-head arc that must be changed can be estimated as follows.

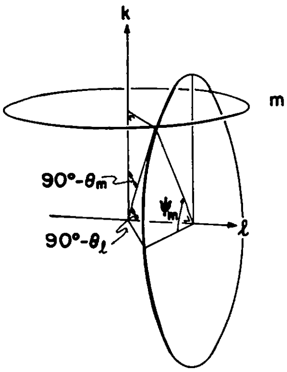

Fig. 3. Relation among ψ_m, θ_m and $\theta_{\mathbf{l}}$. The opening angle of the diffraction cone of **l** is $90°-\theta_{\mathbf{l}}$ and that of **k** is $90°-\theta_m$. The vector **l** is perpendicular to the vector **k** by definition. Note also that the diffraction plane of **l** at $\psi_{\mathbf{l}}=0$ is perpendicular to the plane that contains the vectors **k** and **l**. This is because the goniometer-head arc movement is assumed to be entirely within the plane of the χ-circle.

Suppose that initially, when the reflection **l** diffracts at $\chi=0$ and $\psi=0$, one of the goniometer-head arcs is in the χ circle and that this arc is then moved by an angle $-\Delta$. Reflection **l** will now diffract at $\chi=\chi_0=\Delta$, $\psi=0$ and all other angles unchanged. Let θ_m be the Bragg angle of the reflection **k** at the highest level m. Let $\theta_{\mathbf{l}}$ be the Bragg angle for the reflection **l**. The ψ scan around **l** needs be done only over the range such that its ψ-scan circle crosses that of reflection **k** at level m. Let the absolute value of $\psi_{\mathbf{l}}$ at this intersection be ψ_m. The minimum range of necessary $\psi_{\mathbf{l}}$ values is then from $-\psi_m$ to ψ_m. Referring to Fig. 3, we have

$$\sin\psi_m = \sin\theta_m/\cos\theta_{\mathbf{l}}\,. \tag{16}$$

The maximum value of ω for this range of ψ scan is obtained from (see Hamilton, 1974)

$$\tan(\omega_m-\theta_{\mathbf{l}}) = \sin\psi_m/\tan\chi_0\,. \tag{17}$$

Combining (16) and (17) and recognizing that $\chi_0=\Delta$,

$$\tan\Delta = \sin\theta_m/[\cos\theta_{\mathbf{l}}\tan(\omega_m-\theta_{\mathbf{l}})]\,. \tag{18}$$

For a data set with the nominal resolution of 2·0 Å, $\theta_m=22{\cdot}71°$ with Cu $K\alpha$ radiation. For the Syntex $P\bar{1}$ diffractometer, the maximum value of ω attainable is $\omega_m=58°$. If a secondary reference reflection can be found at $2\theta_{\mathbf{l}}\leq 10°$, which will usually be the case, (18) gives $\Delta\leq 16{\cdot}3°$. Such a change in goniometer-head arc is usually possible. Even for a 1·5 Å data set $\Delta\leq 21{\cdot}2°$, which is still attainable if the arc was somewhat off from the exact zero before the change.

Although these changes in the goniometer-head arc are possible, they are nonetheless large and may make the crystal orientation unstable. On the other hand, the level scale factors need not be determined often. In most cases one set of determinations at the end of data collection will suffice. In addition a 'scan' in ψ is not necessary – one can calculate the necessary ψ values from equations given in the following section and measure intensities at these particular ψ values only.

3. *Calculation of* ψ

(15) contains one ψ angle for each of the four vectors **k**, $\bar{\mathbf{k}}$, **l**, and **l**′ at each level. These angles can be calculated from the diffractometer setting angles for these reflections at $\psi=0$. Actually, there are two sets of ψ angles possible – one corresponding to the tetrahedron of Fig. 2 and another corresponding to its mirror image. These two solutions are given by (see Appendix § 3 for derivation):

$$\psi_{\mathbf{p}} = \psi^0_{\mathbf{p}} \mp \varepsilon_{\mathbf{p}}\ (\text{modulo }\pi) \tag{19}$$

$$\cos\psi_{\mathbf{p}}^0=\left|\frac{\cos\chi_{\mathbf{s}}}{\sin\chi_{\mathbf{p}}}\right|\cos(\varphi_{\mathbf{s}}-\varphi_{\mathbf{p}}) \qquad (20)$$

$$\sin\varepsilon_{\mathbf{p}}=\sin\theta_{\mathbf{s}}/\cos\theta_{\mathbf{p}} \qquad (21)$$

$$\psi_{\mathbf{s}}=\psi_{\mathbf{s}}^0\pm\varepsilon_{\mathbf{s}}\ (\text{modulo }\pi) \qquad (22)$$

$$\cos\psi_{\mathbf{s}}^0=\left|\frac{\cos\chi_{\mathbf{p}}}{\sin\chi_{\mathbf{s}}}\right|\cos(\varphi_{\mathbf{p}}-\varphi_{\mathbf{s}}) \qquad (23)$$

$$\sin\varepsilon_{\mathbf{s}}=\sin\theta_{\mathbf{p}}/\cos\theta_{\mathbf{s}}\,. \qquad (24)$$

In these equations, $\mathbf{p}$ is either $\mathbf{k}$ or $\bar{\mathbf{k}}$ and $\mathbf{s}$ is either $\mathbf{l}$ or $\mathbf{l}'$. One solution is obtained by choosing upper or lower signs in (19) and (22) depending respectively on whether a positive ψ rotation moves the crystal anticlockwise or clockwise when the viewing direction is from the tip of the diffraction vector towards the crystal. For the Syntex $P\bar{1}$ system, this depends on whether the angle χ for the reflection concerned is positive (upper sign) or negative (lower sign) at $\psi=0$. The combination of signs not used in the first solution then gives the second solution. In practice, we prefer to use both solutions in order to increase the accuracy of the scale-factor determination. If a geometrical average of the two solutions is taken, ensurance of proper sign combination becomes unimportant (see the last section). When ψ^0 angles are calculated with (20) and (23), proper branches of the cosine function must be chosen. A criterion is that $\psi_{\mathbf{p}}^0$ and $\varphi_{\mathbf{s}}-\varphi_{\mathbf{p}}$ (and similarly $\psi_{\mathbf{s}}^0$ and $\varphi_{\mathbf{p}}-\varphi_{\mathbf{s}}$) should belong to the same or different quadrant depending on whether a positive ψ rotation increases or decreases the φ reading respectively. For the Syntex $P\bar{1}$ system, these angles should always be in different quadrants. (21) and (24) reflect the fact that, when $\sin\theta_{\mathbf{p}}$ is greater than $\cos\theta_{\mathbf{s}}$, the diffraction cones of $\mathbf{p}$ and $\mathbf{s}$ do not intersect [see equation (16)]. The angle ε ranges from 0 to 90°. It should also be noted that the intensity at a given ψ angle used here is, in general, not the same as that at the same value of ψ before the goniometer-head arc movement. This means that the intensities of the $\mathbf{k}$ reflections must be measured again at these ψ-angles even though a complete ψ scan has been made around these reflections before the goniometer-head arc movement.

Determination of μR and S

The factor S can be looked up from the spherical-transmission-factor table (Bond, 1967) if μR is known. For most small molecule crystals, a fairly accurate estimate of this quantity can readily be made, However, for protein crystals mounted in a capillary tube with an undetermined amount of adhering mother liquor around, a good estimation of this quantity is difficult to make. The following procedure is suggested.

Using a pencil X-ray beam, whose width is smaller than the size of the crystal, one measures the intensities I and I_0 with and without the crystal in the path of the beam. μR may then be estimated from

$$I/I_0=\exp(-\mu R)\,. \qquad (25)$$

One should measure I with the crystal in many different orientations and take the average. It is not clear what kind of average will be most suitable. A procedure consistent with the product approximation will be to take the geometrical mean of the intensities, which is equivalent to taking the arithmetic mean of μR.

Use of the direct beam to estimate the transmission factor of a crystal is, in general, inadequate for at least two reasons. Firstly, the size of the X-ray beam used for data collection is usually larger than that of the crystal, in which case a significant and varying proportion of the direct beam will arrive at the detector without passing through the absorbing matter. This will give rise to a varying degree of underestimation of the absorption factor for the sample. Use of a narrow beam will avoid this problem but then one must assume that the absorption effect suffered by a wide beam is the same as that suffered by a narrow beam. This is a questionable assumption. These difficulties would, however, not be applicable when a narrow beam is used only to estimate effective μR.

A second source of potential difficulty is that the direct beam may pass through a portion of the absorbing matter that the normally diffracting X-ray beam either does not pass through or effectively bypasses. One mechanism whereby this could occur can be seen by considering a large spherical crystal of high absorption coefficient. A normally diffracting beam effectively bypasses the portion of such crystal that is on the backside of the diffraction vector, making the transmission factor increase with increasing θ. A direct beam passes through all portions of the crystal and hence its transmission will be low and constant. If the shape of the crystal is not spherical, this effect will be even more pronounced. This feature again should have no effect when the direct beam is used to estimate effective μR instead of the transmission factors themselves. There is, however, another mechanism that may operate when there is foreign absorbing matter around the crystal. When this foreign matter is on the backside of the diffraction vector it will not absorb the normally diffracting beam but the direct beam will always be affected. This feature will affect even the determination of effective μR but the effect will tend to cancel out if a narrow beam is used and an average is taken over a wide range of crystal orientations.

Experimental procedure

For practical applications, it is convenient to transform (6) and (15) into the following forms.

$$I_{\mathbf{h}}^0=\frac{(I_{\mathbf{h}}I_{\mathbf{h}'})^{1/2}}{[T_k(\varphi_1)T_k(\varphi_2)]^{1/2}S_{\mathbf{h}}} \qquad (26)$$

$$T_k(\varphi_i)=P_k(\varphi_i)Q_k \qquad (27)$$

$$P_k(\varphi_i)=I_{\mathbf{k}}(\varphi_i)/\bar{I}_{\mathbf{k}} \tag{28}$$

$$Q_k=\frac{\bar{I}_{\mathbf{k}}}{S_{\mathbf{k}}I^0_{\mathbf{k}}}=\left(\frac{r_{\mathbf{k}}}{r_{\mathbf{k}_0}}\right)^{1/2}\frac{q_{k_0}}{q_k}\,\frac{\bar{I}_{\mathbf{k}}}{\bar{I}_{\mathbf{k}_0}}\,\frac{\bar{I}_{\mathbf{k}_0}}{S_{\mathbf{k}_0}I^0_{\mathbf{k}_0}} \tag{29}$$

$$q_k=\left[\frac{I_{\mathbf{k}}(\psi^1_{\mathbf{k}})I_{\mathbf{k}}(\psi^2_{\mathbf{k}})I_{\bar{\mathbf{k}}}(\psi^1_{\bar{\mathbf{k}}})I_{\bar{\mathbf{k}}}(\psi^2_{\bar{\mathbf{k}}})}{I_{\mathbf{l}}(\psi^1_{\mathbf{l}})I_{\mathbf{l}}(\psi^2_{\mathbf{l}})I_{\mathbf{l}'}(\psi^1_{\mathbf{l}'})I_{\mathbf{l}'}(\psi^2_{\mathbf{l}'})}\right]^{1/4}. \tag{30}$$

In (26), $I_{\mathbf{h}}$ and $I_{\mathbf{h}'}$ are the intensities of a general reflection $\mathbf{h}=hkl$ and its twofold symmetry equivalent $\mathbf{h}'$ measured in the usual bisecting mode and $S_{\mathbf{h}}$ is the spherical transmission factor that depends only on the value of μR and the Bragg angle $\theta_{\mathbf{h}}$. φ_1 and φ_2 are as given by (7) and (8). In (27) and (28), $i=1$ or 2 and $\bar{I}_{\mathbf{k}}$ is the intensity of the reflection $\mathbf{k}=0k0$ averaged over all φ angles. Q_k is the level scale factor and (29) and (30) are obtained from (15) by incorporating both solutions of ψ angles. The two solutions, indicated by superscripts 1 and 2 for each of the four reflections involved, are obtained by using both of the double signs in (19) and (22). The last factor in (29), $\bar{I}_{\mathbf{k}_0}/S_{\mathbf{k}_0}I^0_{\mathbf{k}_0}$, is a constant and, since only relative values of Q_k need be determined, this factor may be arbitrarily set to unity and dropped from the equation.

The procedure we have set up works as follows. The crystal is mounted in the capillary such that the crystallographic twofold axis (**b** axis) is parallel to the instrument φ axis. Intensity data are collected in the usual bisecting mode. Both members of the twofold-symmetry-related pairs are included in the data set. A series of reflections $\mathbf{k}=0k0$ is selected that is representative of all the k levels of the data set. Intensities from a complete 0 to 360° φ scan are collected in appropriate intervals of φ for each of these reflections at the beginning and at the end of the data collection and also during the data collection at convenient intervals. After all data collection is complete, a couple of reflections of the type $\mathbf{l}=h0l$ are selected that are approximately prependicular to one another and diffract strongly within a 2θ value of about 10°. For each of these reflections, the top stage and the arcs of the goniometer head are rotated until the plane that contains this reflection and the **b** axis coincides with the χ circle and until the ψ scan about the reflection is physically possible within the range from $-\psi_m$ to ψ_m as calculated by (16). The orientation matrix of the crystal is then re-determined and the intensities of reflections $\mathbf{k}$, $\bar{\mathbf{k}}$, $\mathbf{l}$, and $\mathbf{l}'$ are measured at the set of ψ angles calculated by (19) to (24). After this procedure is completed for both of the **l**-type reflections, the X-ray generator setting is decreased to reduce the intensity of the X-ray beam, the beam collimator is replaced with one of narrow aperture, the beam stop is removed and the intensity of the direct beam is measured at many different crystal orientations. Finally, the crystal is removed and the intensity of the direct beam is measured again.

Absorption correction is made after background correction (Krieger, Chambers, Christoph & Stroud, 1974) but before decay correction. The reason that absorption correction should be made before decay correction is that the intensity of the reflections used to monitor the crystal decay may be affected by the change in the absorption characteristics of the sample caused by slight rotation of the crystal and movement of the surrounding mother liquor.

The φ scan intensities of each reflection **k** are first averaged over the pairs that are 180° apart from one another in φ and then divided by the average over all φ angles. These averaged, normalized φ scan intensities are the $P_k(\varphi)$ values of (27) and (28). The level scale factors Q_k are computed with (29) (with $r_{\mathbf{k}}$ and $\bar{I}_{\mathbf{k}_0}/S_{\mathbf{k}_0}I^0_{\mathbf{k}_0}$ set to unity) and (30). We obtain two sets of Q_k, one for each **l**-type reflection chosen, and take the geometrical average of the two.

$\bar{I}_{\mathbf{k}}$ and $\bar{I}_{\mathbf{k}_0}$ used in (29) should be the average of intensities measured in the last *complete* ψ scan. This procedure implicitly assumes that the level scale factors $Q_k=\bar{I}_{\mathbf{k}}/S_{\mathbf{k}}I^0_{\mathbf{k}}$ are not affected by decay or by movement of the crystal and mother liquor relative to the glass capillary during the data collection. There is no reason why $\bar{I}_{\mathbf{k}}/S_{\mathbf{k}}I^0_{\mathbf{k}}$ should be affected by decay as long as the ψ scan interval is chosen fairly small. On the other hand, it will be affected by the movement of the crystal and mother liquor relative to the glass capillary since the χ dependence of the absorption may change. The level scale factors should, however, be rather insensitive to this movement. If large movement has occurred, there will be no way to recover proper scale factors and if the χ dependence of the absorption correction is large, the data set will have to be re-collected.

A table of $T_k(\varphi)$ values is then set up which contains as many rows as the number of ψ scans performed around all **k** and as many columns as the number of φ values used in the scans. For a given general reflection **h**, the two φ values, φ_1 and φ_2, are computed with (7) and (8). The $T(\varphi_1)$ and $T(\varphi_2)$ values are then obtained by two-dimensional linear interpolation of the table values. The one or two rows of the table used in this interpolation should be those of the ψ scans that had been made nearest in time to the measurement of **h** and bracket the k level of **h**.

If the intensity of **k** is low, the $P_k(\varphi)$ curve will be jagged. In such a case, the experimental values are fitted to a polynomial of trigonometric functions of φ and the calculated smooth curve should be used instead of the raw experimental values. If the intensities of **l** are low, the calculated Q_k values may also be erratic. In such a case, Q_k should also be fitted to a polynomial of k or of trigonometric functions of χ. In fact it may be desirable to fit the entire table of T to a polynomial of the trigonometric functions of φ and χ. We are in the process of developing proper functional forms to use for this purpose.

When the two T values are obtained either by table look-up or by the analytical function developed above, a geometrical average is taken and the intensity of the reflection is then divided by this number. The spherical absorption correction is then applied by looking up the value of S from the spherical transmission factor table with the θ value of $\mathbf{h}$ and the μR computed from the intensities of the direct beam according to (25) and averaged over all crystal orientations. The geometrical average over the symmetry equivalents $\mathbf{h}$ and $\mathbf{h}'$ is taken at a convenient later point during the chain of data-reduction process.

Obviously it is desirable that this program of absorption correction be tested on real crystals. A computer program has been written in Fortran and is available from our laboratory. The results of experimental tests will be reported later.

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APPENDIX

1. *A useful trigonometric formula*

In the following two sections, a trigonometric formula will be used to derive angular relations from inspection of appropriate drawings. This formula is described here. Let **1**, **2**, and **3** be any three vectors. Let α_1, α_2, and α_3 be the angles between **2** and **3**, **1** and **3**, and **1** and **2**, respectively. Then the angle ω between two planes – one that contains vectors **1** and **2** and the other that contains vectors **1** and **3** – is given by

$$\cos\omega=\frac{\cos\alpha_1-\cos\alpha_2\cos\alpha_3}{\sin\alpha_2\sin\alpha_3}. \tag{A1}$$

Derivation of this formula is simple and need not be given here. One should note that, if the vectors are along the crystallographic axes, then α_1, α_2, and α_3 are the three angles of the unit cell and ω is the complement of the reciprocal angle α_1^*. When use is made of this formula, the three vectors **1**, **2**, and **3** will be indicated in that order.

2. *Calculation of ψ angles in the bisecting geometry*

Consider the following operations. First, set the diffractometer such that a general reflection $\mathbf{h}=hkl$ is in the diffracting position in the bisecting mode. At this setting the diffractometer angles read $\omega_\mathbf{h}(=\theta_\mathbf{h})$, $\chi_\mathbf{h}$, and $\varphi_\mathbf{h}$. Let $\mathbf{I}$ and $\mathbf{R}$ denote the vectors along the incident and reflected beams. The plane that contains the φ axis and the diffraction vector $\mathbf{h}$ at this position is coincident with the χ circle, is perpendicular to the diffraction plane of $\mathbf{h}$ (the plane that contains $\mathbf{h}$, $\mathbf{I}$, and $\mathbf{R}$), and bisects $\mathbf{I}$ and $\mathbf{R}$.

Now suppose that the vectors $\mathbf{I}$ and $\mathbf{R}$ are fixed to the crystal and that the crystal and the two beam directions are rotated as one unit until the diffractometer angles read $\omega=0°$, $\chi=\chi_0$, and $\varphi=\varphi_0$. χ_0 and φ_0 are the χ and φ angles where the reflection $\mathbf{k}=0k0$ diffracts at $\psi=0°$. Since the crystal is mounted such that the $\mathbf{b}^*$ axis is parallel to the φ axis, χ_0 equals 90 or 270°.

Fig. 4 illustrates the geometries involved at this crystal orientation. The plane that contains the diffraction vectors $\mathbf{h}$ and $\mathbf{k}$ is still perpendicular to the diffraction plan of $\mathbf{h}$ and bisects the vectors $\mathbf{I}$ and $\mathbf{R}$. The angle between the plane of the χ circle and the plane that contains $\mathbf{h}$ and the φ axis is $\Delta\varphi$, the amount of φ rotation necessary to make the reflection $\mathbf{h}$ diffract in the bisecting mode. Inspection of Fig. 4 gives

$$\varrho_\mathbf{h}=|\chi_\mathbf{h}-\pi/2| \text{ or } |\chi_\mathbf{h}-3\pi/2| \tag{A2}$$

$$\varrho_\mathbf{I}=\varrho_\mathbf{R} \tag{A3}$$

$$\cos\varrho_\mathbf{R}=\cos(\pi/2-\theta_\mathbf{h})\cos\varrho_\mathbf{h} \tag{A4}$$

$$\sigma_\mathbf{h}=\sigma_0\pm\Delta\varphi \tag{A5}$$

$$\sigma_\mathbf{I}=\sigma_\mathbf{h}-\Delta\sigma \tag{A6}$$

$$\sigma_\mathbf{R}=\sigma_\mathbf{h}+\Delta\sigma \tag{A7}$$

$$\Delta\varphi=\varphi_\mathbf{h}-\varphi_0 \tag{A8}$$

$$\cos\Delta\sigma=\frac{\cos(\pi/2-\theta_\mathbf{h})-\cos\varrho_\mathbf{h}\cos\varrho_\mathbf{R}}{\sin\varrho_\mathbf{h}\sin\varrho_\mathbf{R}} \tag{A9}$$

$$|\mathbf{h}|\cos\varrho_\mathbf{h}=|k|b^* \tag{A10}$$

$$\cos\varrho_\mathbf{R}=|k|\lambda b^*/2. \tag{A11}$$

In these equations, ϱ is the angle that a given vector ($\mathbf{h}$, $\mathbf{I}$, or $\mathbf{R}$) makes with the vector $\mathbf{k}$, σ is the azimuthal angle of the vector measured from a certain reference point such as the b^*c^* plane, and σ_0 is the σ angle of the plane of the χ-circle. The sense of the σ angle is such that a positive σ rotation moves a right-handed screw along the positive direction of $\mathbf{k}$. $\Delta\sigma$ ranges

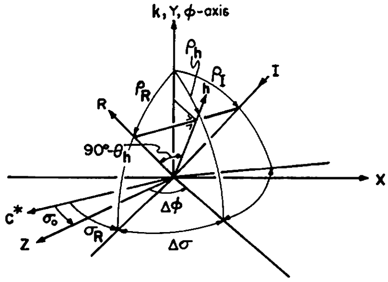

Fig. 4. Orientation of the incident (**I**) and the reflected (**R**) beam directions of a reflection $\mathbf{h}=hkl$ after they are fixed to the crystal and rotated with it until the diffractometer is at the position where the reference reflection $\mathbf{k}=0k0$ diffracts at $\psi_\mathbf{k}=0$. At this position χ is 90 or 270°. The y axis coincides with the φ axis and also with the vector $\mathbf{k}$, the x axis coincides with the χ axis and the χ circle lies on the yz plane. The incident and reflected beams are on the xy plane and the detector moves in the same plane.

from 0 to 90°. ($A9$) is obtained through the application of ($A1$) on vectors $\mathbf{k}$, $\mathbf{h}$, and $\mathbf{R}$. ($A11$) is obtained by combining ($A4$), ($A10$) and Bragg's law: $|\mathbf{h}| = 2\sin\theta_{\mathbf{h}}/\lambda$.

The ambiguities expressed in ($A2$) and ($A5$) require some explanation. In general, there are eight different diffractometer settings, at each of which the diffraction of a given reflection can be observed with the χ circle in a position bisecting the incident and reflected beams (Hamilton, 1974). The data collection software of a particular diffractometer system chooses one of these settings for each reflection using certain criteria which may vary from system to system. The ambiguities indicated in ($A2$) and ($A5$) are due to these different possibilities of diffractometer setting and also to the different sign conventions used concerning the sense of rotation of various angles. The proper form of ($A2$) to use is the one that gives a $\varrho_{\mathbf{h}}$ value in the range from 0 to 90°. In ($A5$), upper and lower signs should be used respectively, depending on whether the instrument φ reading increases or decreases when the crystal is viewed from the positive end of the vector $\mathbf{k}$ towards the origin and rotated clockwise. Even when the correct sign is used, ($A5$) can be incorrect by 180° depending on which diffractometer setting is used but, as will be seen later, this ambiguity is of no practical consequence for the purpose of this paper. ($A6$) and ($A7$) are correct only for certain diffractometer settings. For other settings, the subscripts $\mathbf{I}$ and $\mathbf{R}$ must be interchanged. This ambiguity is again of no practical consequence.

Consider two reflections $\mathbf{h}$ and $\mathbf{h}'$ that are related to one another by a twofold rotation symmetry about $\mathbf{k}$. Clearly $\theta_{\mathbf{h}} = \theta_{\mathbf{h}'}$, $\varrho_{\mathbf{h}} = \varrho_{\mathbf{h}'}$, and $\sigma_{\mathbf{h}} = \sigma_{\mathbf{h}'} + \pi$. ($A3$) and ($A4$) then show that the incident and reflected beam directions of these reflections, *i.e.* the vectors $\mathbf{I}$, $\mathbf{R}$, $\mathbf{I}'$ and $\mathbf{R}'$, all have the same ϱ. In fact, the X-ray beams of all reflections with a common k index lie on the surface of one cone whose opening angle is $\varrho_{\mathbf{R}}$ as given by ($A11$). This is of course a well-known fact from the equi-inclination Weissenberg geometry. In addition, ($A6$) and ($A7$) show that the σ angles of $\mathbf{I}'$ and $\mathbf{R}'$ are 180° apart from those of $\mathbf{I}$ and $\mathbf{R}$. The four vectors $\mathbf{I}$, $\mathbf{R}$, $\mathbf{I}'$, and $\mathbf{R}'$ therefore conform to the twofold symmetry as do $\mathbf{h}$ and $\mathbf{h}'$. This is a characteristic property that one obtains when the crystal is mounted such that the symmetry axis is parallel to the φ axis and the data are collected in the bisecting mode. If either of these conditions is not met, this desirable property does not obtain (see the accompanying paper).

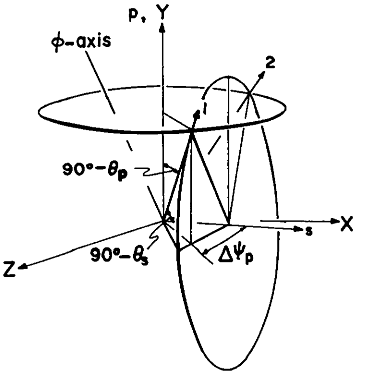

Fig. 5. Geometry of the intersection of two orthogonal diffraction cones when $\mathbf{p}$ is in the diffracting position at $\psi_{\mathbf{p}} = 0$. The incident and reflected beams are in the xy plane. The χ circle is in the yz plane and bisects the incident and reflected beams. The φ axis lies on this plane. The angle between s and the z axis is $|\psi_{\mathbf{p}}^0|$. The vectors $\mathbf{1}$ and $\mathbf{2}$ run along the intersections of the two diffraction cones. The projections of these vectors on the xz plane make the angles $|\psi_{\mathbf{p}}^1|$ and $|\psi_{\mathbf{p}}^2|$, respectively, with the x axis and an angle $\Delta\psi_{\mathbf{p}}$ with the vector s.

These properties – that the four ray directions $\mathbf{I}$, $\mathbf{R}$, $\mathbf{I}'$ and $\mathbf{R}'$ lie on the surface of the diffraction cone of $\mathbf{k}$ and that they conform to the twofold rotation symmetry about $\mathbf{k}$ – clearly ensure that it is always possible to choose two φ angles at which the incident and reflected beam directions of $\mathbf{k}$ coincide with these four beam directions. Inspection of Fig. 4 shows that these angles are given by

$$\varphi = \varphi_{\mathbf{h}} \pm \Delta\sigma + \pi/2 \text{ (modulo } \pi\text{)}. \qquad (A12)$$

This equation can be converted to (7) and (8) of the main text by a somewhat lengthy but straightforward mathematical transformation using equations ($A2$), ($A4$), and ($A9$) and introducing a new variable $\varepsilon = \pi/2 - \Delta\sigma$.

3. *Intersection of two mutually orthogonal diffraction cones*

Let $\mathbf{p}$ and $\mathbf{s}$ be any mutually orthogonal pair of reflections, neither of which is necessarily on the φ axis. Turn the diffractometer until one of them, say $\mathbf{p}$, is in the diffracting position at $\psi = 0$. Fig. 5 shows the geometry at this crystal orientation. The χ circle lies on the yz plane. The φ axis also lies on this plane. The χ axis is along the x axis and the incident and reflected beams lie on the xy plane. Assuming that the two diffraction cones meet, the intersections define the two vectors $\mathbf{1}$ and $\mathbf{2}$.

Now fix the coordinate system and the incident and reflected X-ray beams in space and turn the diffractometer to rotate the crystal about the vector $\mathbf{p}$. This is the ψ rotation about $\mathbf{p}$. Let $\psi_{\mathbf{p}}^1$ and $\psi_{\mathbf{p}}^2$ be the two ψ angles when the vectors $\mathbf{1}$ and $\mathbf{2}$, respectively, are along the incident or reflected beam. These are the positions when the vector $\mathbf{1}$ or $\mathbf{2}$ is in the xy plane. Let $\psi_{\mathbf{p}}^0$ be the ψ angle when the vector $\mathbf{s}$ is coincident with the z axis.

These ψ angles can be calculated from the diffractometer setting angles of $\mathbf{p}$ and $\mathbf{s}$ as follows. Let θ, φ, and χ with subscripts $\mathbf{p}$ or $\mathbf{s}$ be the Bragg diffraction angle and the diffractometer setting angles when $\mathbf{p}$ or $\mathbf{s}$ diffracts in the bisecting mode. One notes that the angle between the φ axis and the vector $\mathbf{p}$ is $|90° + \chi_{\mathbf{p}}|$ or $|90° - \chi_{\mathbf{p}}|$ depending on the sign convention of the sense of rotation of χ and on the position of the bisecting mode (see Appendix 2). We shall call this angle $\chi_{\mathbf{p}}'$. Similarly the angle between the φ axis and the vector **s** will be called χ'_s and is equal to $|90° + \chi_s|$ or $|90° - \chi_s|$. One can then write the following equations directly by an inspection of Fig. 5.

$$\psi^i_p = \psi^0_p + \pi/2 \pm \Delta\psi_p \text{ (modulo } \pi) \qquad (A13)$$

$$\cos(\varphi_s - \varphi_p) = \frac{\cos \pi/2 - \cos \chi'_p \cos \chi'_s}{\sin \chi'_p \sin \chi'_s} \qquad (A14)$$

$$\cos \psi^0_p = \frac{\cos \chi'_s - \cos \chi'_p \cos \pi/2}{\sin \chi'_p \sin \pi/2} \qquad (A15)$$

$$\cos(\pi/2 - \theta_s) = \sin(\pi/2 - \theta_p) \cos \Delta\psi_p\,. \qquad (A16)$$

In (A13), the double sign is for $i=1$ or 2. (A14) and (A15) are obtained by application of (A1) on vectors φ axis, **p** and **s** and on vectors **p**, φ axis and **s** respectively. With the introduction of a new variable $\varepsilon_p = \pi/2 - \Delta\psi_p$, these equations can be rewritten as

$$\psi^i_p = \psi^0_p \pm \varepsilon_p \text{ (modulo } \pi) \qquad (A17)$$

$$\cos(\varphi_s - \varphi_p) = \pm \frac{\sin \chi_p \sin \chi_s}{\cos \chi_p \cos \chi_s} \qquad (A18)$$

$$\cos \psi^0_p = \pm \frac{\sin \chi_s}{\cos \chi_p} = \pm \frac{\cos \chi_s}{\sin \chi_p} \cos(\varphi_s - \varphi_p) \qquad (A19)$$

$$\sin \varepsilon_p = \frac{\sin \theta_s}{\cos \theta_p}\,. \qquad (A20)$$

It can be shown by considering all possible sign conventions and diffractometer settings that the ambiguity in sign in (A19) should be removed by requiring that $\varphi_s - \varphi_p$ and ψ^0_p belong to the same or different quadrants depending respectively on whether the senses of the φ and ψ rotations are the same or different.

This set of equations can be written for each of the four vectors **k**, $\bar{\mathbf{k}}$, **l** and **l**′. These give all the ψ angles necessary for all eight vectors of intersection, from which proper sets of four vectors must be selected according to Fig. 2. These considerations give (19) to (24) and the rules for selecting proper signs and branches of the cosine function as stated with these equations in the main text.

References

BOND, W. L. (1967). *International Tables for X-ray Crystallography*, Vol. II, 2nd ed., pp. 300–305. Birmingham: Kynoch Press.

FLACK, H. D. (1974). *Acta Cryst.* A**30**, 569–573.

FURNAS, T. C. (1957). *Single Crystal Orienter Instruction Manual.* General Electric Company, Milwaukee.

HAMILTON, W. C. (1974). *International Tables for X-ray Crystallography*, Vol. IV, pp. 276–279. Birmingham: Kynoch Press.

KATAYAMA, C., SAKABE, N. & SAKABE, K. (1972). *Acta Cryst.* A**28**, 293–295.

KOPFMANN, G. & HUBER, R. (1968). *Acta Cryst.* A**24**, 348–351.

KRIEGER, M., CHAMBERS, J. L., CHRISTOPH, G. G. & STROUD, R. M. (1974). *Acta Cryst.* A**30**, 740–748.

LEE, B. & RUBLE, J. R. (1977). *Acta Cryst.* A**33**, 637–641.

NORTH, A. C. T., PHILLIPS, D. C. & MATHEWS, F. S. (1968). *Acta Cryst.* A**24**, 351–359.

Acta Cryst. (1977). A**33**, 637–641

A Semi-empirical Absorption-Correction Technique for Symmetric Crystals in Single-Crystal X-ray Crystallography. II

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This is the second part of a two-part series. The first part described the technique in the case when the symmetry axis is coincident with the instrument φ axis. This paper describes the procedure to follow when the orientation of the symmetry axis is arbitrary.

Introduction

In the previous paper (Lee & Ruble, 1977), a procedure for the semi-empirical absorption correction has been presented for the case where a crystallographic twofold axis is coincident with the instrument φ axis. However, there are cases where aligning a symmetry axis along the φ axis presents serious practical problems. This will be the case if the crystal is so ill-formed that the crystallographic symmetry axis is impossible to identify under microscopic examination. In the case of protein crystals, which have to be mounted in capillary tubes, the difficulty also arises if the shape of the crystal is very anisotropic and also such that the symmetry axis runs along the short dimension of the crystal. The crystal, in this case, will tend to orient itself such that its long dimension is parallel to the capillary axis, making it difficult to