A Simple Empirical Absorption-Correction Method for X-ray Intensity Data Films

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The transmission of an incident X-ray beam in a particular direction depends on the absorption properties of the crystal in this direction. A measurement of the relative transmission of the incident beam in all possible directions allows, therefore, in principle, the determination of the three-dimensional absorption surface of the crystal. The relative transmission factor of the incident beam for all directions of interest can easily be determined on a precession or rotation camera if the beam cross section is smaller than the crystal size. The absorption surface determined in this way may be used to correct X-ray intensity films obtained under similar geometrical conditions, *i.e.* screenless precession or rotation photographs. The method was tested by its effect on the symmetry averaging reliability index of a large number of reflexions from various films of various crystals. This value decreased typically from 8·2 to 6·1 % after application of the absorption correction, indicating that the correction accounts for a large part of the absorption effect.

Introduction

Film methods of data collection are becoming increasingly important in protein crystallography. Oscillation, rotation (Arndt, 1968, 1973) and precession techniques (Xuong & Freer, 1971) without use of layer-line screens appear to be very efficient methods as all reflexions traversing the Ewald sphere are sampled. However, overlap of reflexions and edge effects are a serious drawback in these techniques. The proportion of reflexions which are overlapped or lie on edges increases with cell size of the crystal lattice and spot size of the reflexions.

A very simple and effective means of reducing the spot size is to reduce the cross fire of the primary beam by a narrow pin-hole system. The cross section of the beam will then usually be smaller than the crystal size.

As the rotation or precession angle is normally small ($\sim 1·5°$), intensity variations due to volume effects appear to be negligible, but absorption effects remain. These effects may even be worse than in the case of a crystal completely bathed in the X-ray beam as can easily be visualized in the case of a mis-centred spherical crystal.

If the crystal is completely bathed in the X-ray beam, an experimental determination of the absorption surface is possible by diffractometer measurements. The absorption surface (North, Phillips & Matthews, 1968; Kopfmann & Huber, 1968) may then be used to correct the photographic data. Such an absorption correction is not possible with partially irradiated crystals since the transfer from the X-ray camera to a diffractometer, maintaining exactly the same conditions, appears impossible.

Methods

(a) Minimization of absorption effects

If the crystal has boundary planes perpendicular to the primary beam and if it is of large size compared with the primary-beam cross section, the absorption effects are small. Absorption for reflexions within a certain diffraction cone does not vary and the variation between different cones is not large for low-resolution data as is usual in protein crystallography. Furthermore this effect may easily be corrected by a film-scaling procedure including individual 'temperature factors' (Steigemann, unpublished). The situation is demonstrated in Fig. 1(a) and 1(b). A crystal setting as shown in Fig. 1(a) shows much a smaller absorption effect than the setting in Fig. 1(b), where the X-ray paths are different at the left and right-hand sides. It is therefore desirable to use a particular crystal in a setting with two parallel faces perpendicular to the primary beam if possible. Such settings may be found by microscopic inspection of the crystal. If the crystal has only one face perpendicular to the primary beam, it should be oriented so that this face is at the side opposite to the primary beam. This is demonstrated in Fig. 2(a) and (b). While the setting of Fig. 2(a) produces a small absorption effect, that of 2(b) is unfavourable.

(b) Absorption correction

Kopfmann & Huber (1968) have shown that the transmission factor can be approximated by a product of two partial transmission factors of the primary and secondary beams

$$A_{p,s} \simeq A_p'^p \cdot A_s'^s * \tag{1}$$

where $A_{p,s}$ is the transmission factor for a reflexion with primary-beam direction $\mathbf{p}$ and secondary-beam direction $\mathbf{s}$; $A_p'^p$ and $A_s'^s$ are the partial transmission factors of the primary and secondary beams respectively. This formula is in principle also valid for the unreflected transmission of an X-ray beam. Here $\mathbf{p}$ and $\mathbf{s}$ have the same direction (Kopfmann, 1972). The

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* The nomenclature differs from that used by Kopfmann & Huber (1968), but is clearer.

approximation (1) is excellent for crystals of moderate size and with a moderate linear absorption coefficient. It has been shown (Huber & Kopfmann, 1969) that under certain conditions the partial transmission factor of a primary beam $A_p'^p$ is equal to the partial transmission factor of a secondary beam $A_s'^s$ if p and s are in the same direction,

$$A_p'^p = A_p'^s .   (2)$$

Equation (2) holds exactly for crystals of arbitrary shape without external absorber or with an external absorber having a centrosymmetric distribution of matter. For crystals with non-centrosymmetric external absorbers equation (2) is only approximately valid. The analysis of the absorption surfaces of more than 100 protein crystals by the method of Huber & Kopfmann (1969) showed that the approximation (2) is in general sufficiently fulfilled.

The transmission factor of an unreflected beam may therefore be approximated by

$$A_{p,p} \sim A_p'^p . A_p'^s \sim (A_p'^p)^2 .   (3)$$

Thus the transmission surface can in principle be determined by measuring the transmission of the unreflected primary beam in different orientations of the crystal. Fig. 3 clearly shows that the paths of the individual rays are practically the same for the secondary beam in Fig. 3(a) and the unreflected primary beam at the 'secondary side' in Fig. 3(b). It is also clear that $A_p'^p = A_p'^s$ in Fig. 3(b) as described before.

Such an experiment is of course, not feasible as it would require a primary beam of variable shape exactly identical with the crystal cross section in a particular orientation.

If the crystal cross section is larger than the cross section of the primary beam the experiment seems possible. Under these conditions, however, the validity of the formalism of equations (1) to (3) is unclear. Fig. 4 shows the paths of individual rays of a secondary beam originating from the irradiated volume of a partially irradiated crystal. The dashed lines show the paths of an unreflected primary beam passing through the crystal in an orientation identical with the secondary beam. In contrast to Fig. 3, only parts of the irradiated crystal volumes are the same in the diffraction and the transmission experiments. But the path lengths of the rays of the primary beam at the 'secondary side' appear to represent an average of the path lengths of the rays of the secondary beam. The relative transmission factor of the primary beam is therefore here also expected to be an approximate measure of the relative partial transmission factor of the secondary beam passing the crystal in the same direction. Furthermore, the true geometric situation appears to lie between that shown in Fig. 3 and 4 in general. Evidence that these assumptions are indeed justified comes from the experimental tests to be discussed later.

Under the experimental conditions discussed, the partial transmission factor of the primary beam in the

diffraction experiment is constant for all reflexions. The transmission of the primary beam in the transmission experiment should therefore be proportional to the square of the relative partial transmission factor of the secondary beam in the diffraction experiment.

Experimental

The relative transmission of the unreflected primary beam is easily determined on a precession camera after removal of the primary beam stop. The film holder is displaced so that the primary beam produces a blackened ring on a film during precession motion. Attention must be called to the fact that the crystal and the film holder do not make a uniform precession motion around the primary-beam direction (Buerger, 1964; Waser, 1951). A Lorentz correction factor has
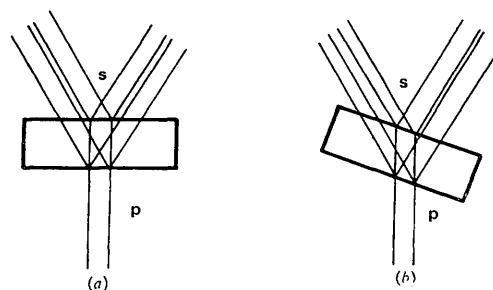

Fig. 1. In setting (b) the absorption of reflexions at the left-hand side is higher than at the right-hand side. The absorption effect is smaller for setting (a).
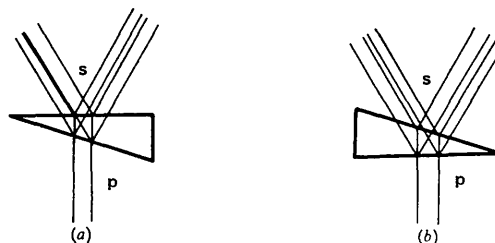

Fig. 2. Setting (a) is more favourable than setting (b); there is an appreciably higher absorption for the left-hand-side reflexions than for the right-hand-side reflexions in setting (b).
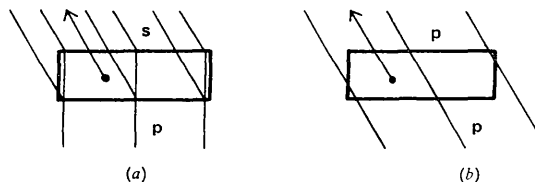

Fig. 3. Essentially the whole crystal volume is irradiated. It is clearly visible that the secondary rays originate from the same volume elements in (a) and (b) except for small areas at the crystal edges. The partial transmissions for the secondary beams in (a) and (b) must therefore be essentially identical.

to be applied to the measured primary-beam intensities. This factor is expressed as:

$$\frac{1}{L} \sim d \cdot \sin \mu(1 + \sin^2 \varphi \cdot \tan^2 \mu)$$

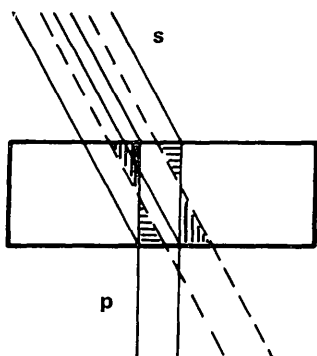$$= r \cdot \cos \mu(1 + \sin^2 \varphi \cdot \tan^2 \mu)$$

Fig. 4. Transmission and diffraction experiment. The irradiated volumes are not identical under the two experimental conditions, but the path-lengths of the secondary rays originating from the excess volumes appear to average out for both experiments. —— X-ray paths in diffraction experiment; ------ X-ray paths in transmission experiment.

where d is the displacement of the holder, μ is the precession angle, r is the radius of the blackened ring and φ the polar angle in the film plane ($\varphi = 0$ corresponds to the horizontal axis).

The correction for oblique incidence of the beam is given by

$$I_{corrected} = I_{observed} \cdot [1 - \exp(-A)]/[1 - \exp(-A \sec \mu)].$$

A is determined by the ratio of intensities of the first and second films in a pack, which is $\exp(-A)$ for perpendicular incidence.

By choosing various precession angles, all desired orientations of the crystal with respect to the primary beam are realized and the corresponding primary beam intensity is recorded on the film. The square root of the intensity variation, corrected for the Lorentz factor, is then used as a correction factor for absorption on the photographs. We found it sufficient to record at settings of μ: 10 and 20° corresponding to reflexion cones with 2θ: 10 and 20° respectively and to make proper inter- and extrapolations for other reflexion angles.

The precession cameras used had a crystal-to-focus distance of 300 mm. The collimator apertures were 0·4 or 0·3 mm. The film-to-crystal distance was 75 mm.
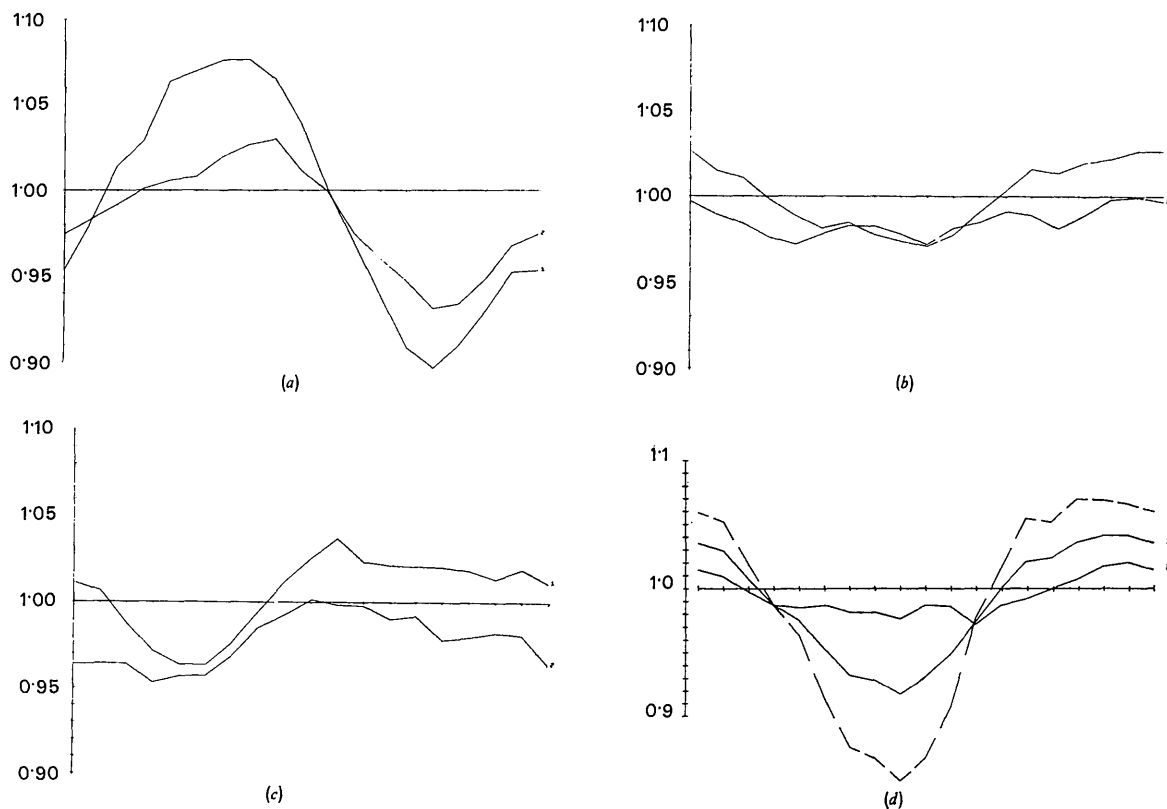

Fig. 5. Absorption curves of four different crystals used for the correction of various films as described in the text. The absorption coefficients to be applied to the structure factor amplitudes are plotted *versus* the polar angle φ. $\varphi = 0$ in the horizontal direction. Curve 1 is obtained from the ring at $\mu = 20°$, curve 2 at 10°. The dashed curve in (d) is the curve at $\mu = 30°$ obtained by extrapolating the 10 and 20° curves.

Graphite-monochromated Cu $K\alpha$ radiation was used. To record the primary beam the tube current was reduced to 2 mA and an attenuator had to be introduced. These absorption photographs were scanned on an Optronics automatic densitometer with a 100μ raster and processed. The intensity values of the raster points on the two absorption rings which were above background were added within segments of $20°$. These averaged intensity values, properly modified as discussed above, served then as absorption correction factors. Fig. 5(a), (b), (c), (d) shows several examples of absorption-correction curves obtained in this way.

The same experimental principles may also be easily applied to the rotation technique if the rotation camera has a tilting stage and transport mechanism for the film holder coupled to the rotation axis.

We wish to emphasize the obvious importance of careful crystal centring on the camera. Furthermore, we found it necessary in some cases to check and to improve the alignment of our commercial precession cameras.

Experimental test

The absorption-correction procedure has been tested on crystals of the trypsin inhibitor–trypsin complex which were usually of irregular shape and larger than $0·5$ mm in their smallest dimension. The space group of these crystals is $I222$ with lattice constants a: $75·7$ b: $84·4$, c: $122·9$ Å (Rühlmann, Schramm, Kukla & Huber, 1971). Diffraction intensities of various crystal specimens prepared from the native protein and heavy-metal derivatives were recorded on screenless precession photographs with a precession angle of $1·5°$. The crystals were oriented so that their c axis coincided with the dial axis of the camera. All X-ray photographs, therefore, showed a mirror line perpendicular to the c axis; those at rotation angles $\alpha = 0$ and $90°$ (a and b axis respectively in the direction of the primary beam) had mm symmetry. Two films in one pack were found sufficient to record all intensities within the optical density region 0 to $2D$, except for a few very strong reflexions. These films were scanned on an Optronics automatic densitometer and processed using the method and programmes of Xuong & Freer (1971) modified by one of us (Schwager, unpublished).

Our test was to compare the deviations of the intensities of symmetry-equivalent reflexions before and after absorption correction.

The statistical measure to be compared was

$$R = \sum \Delta I / \sum I$$

where I is the intensity of a reflexion averaged over the individual measurements and ΔI the deviation of an individual measurements from the mean intensity I. The summation was taken over all reflexions on a single photograph after averaging the data of the two films in one pack, $i.e.$, approximately 1200 reflexions in each case.

We compared the R values of several X-ray photo-graphs from various crystals showing no or very small absorption effects with R values obtained from films with large absorption effects after absorption correction. 15 photographs of the first type showed an average R value of $6·1\%$ with extreme values of $8·0$ and $4·7\%$. 20 photographs of the second type also had an average R value of $6·1\%$ with extreme values of $8·5$ and $3·9\%$. These 20 films had an average R value before absorption correction of $8·2\%$ with extreme values of $12·4\%$ and $4·8\%$. Furthermore, we calculated an analogously defined R value of two photographs obtained from two different crystals in identical settings after scaling the data together. Here the R value decreased from $9·3$ to $7·6\%$ after correction. The summation was over all reflexions on the two film packs, $i.e.$, approximately 2400 reflexions.

This comparison strongly indicates that the correction method is able to account for a large part of the absorption effect. A few examples may illustrate this further.

Fig. 5(a) shows an absorption curve with a strong absorption effect, which is approximately symmetrical around $\varphi = 90$ and $270°$. The two intensities of the 'left–right' pair of symmetry-related reflexions on photographs with rotation angles α different from 0 or $90°$ will therefore be modified by similar correction factors. The R values for these films before and after absorption correction should not change significantly. The photograph at $\alpha = 90°$ however has four equivalent reflexions and the intensities of the 'up-and-down' pairs are strongly affected. The R value for this photograph should drop considerably after application of the correction. Table 1 shows the expected R value changes.

Table 1. *Some characteristic R-value changes of data from various photographs of four different crystals after application of the absorption correction*

The absorption curves used for the corrections are indicated by their Figure numbers. Photographs with differences of up to $10°$ in their spindle dial setting are corrected using the same curve.

The R values were calculated as described in the text with the summation taken over about 1200 reflexions in each case.

Absorption curve Figure	Rotation angle α (°)	R uncorrected (%)	R corrected (%)
5(a)	80	6·3	6·3
	85	6·9	5·3
	90	12·1	6·8
5(b)	0	9·3	6·4
	5	8·3	6·0
	10	8·3	5·4
5(c)	0	10·2	8·0
	10	5·3	5·2
5(d)	85	10·2	4·7

Fig. 5(b) on the other hand, is an absorption curve with an absorption effect approximately symmetrical

around $\varphi = 0$, 180°. This eff ct influences mostly the 'left–right' reflexion pairs and the R value improvement should be observed on all photographs, as indeed shown in Table 1.

The absorption curve of Fig. 5(c) is again approximately symmetrical around $\varphi = 90°$ and 270° and the R value changes are as one would expect.

Fig. 5(d) is an example of a large absorption effect. The R value improvement is particularly high.

This R-value test of equivalent reflexions can, of course, give no indication as to whether the proposed method also provides a proper correction for the absorption variation between diffraction cones; nevertheless it may well do so.

Eventual errors in this correction are, however, no serious problem, as the scaling procedure involves individual scaling factors and temperature factors for each film (Steigemann, unpublished). The 'temperature factor' can take absorption errors of this kind into account.

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Accurate Determination of Atomic Scattering Factors of F.C.C. and H.C.P. Metals by High-Voltage Electron Diffraction

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The accelerating voltage E_c at which the second-order Kikuchi line vanishes has been measured for the 400 reflexions of Al, Ni and Cu and the 0004 reflexion of Ti. The X-ray atomic scattering factors f^x for the first-order reflexion have been determined from the measured values of E_c and the results are compared with theoretical and X-ray experimental data.

Introduction

Two new experimental methods of determining atomic scattering factors have been developed recently. Both methods utilize many-beam dynamical effects in electron diffraction: one uses the effect of the vanishing of the second-order reflexion which occurs due to relativistic enhancement of dynamical interaction among systematic reflexions (Uyeda, 1968; Watanabe, Uyeda & Kogiso, 1968; Watanabe, Uyeda & Fukuhara, 1968); the other uses a similar effect in the accidental many-beam case (Gjønnes & Høier, 1969, 1971). In the former method, the accelerating voltage E_c at which a second-order reflexion vanishes is used to determine the first-order Fourier coefficient, V_1, of the crystal potential; this gives the corresponding value of the X-ray atomic scattering factor f_1^x with high accuracy. The E_c values have been measured so far for the 222

reflexions of f.c.c. metals Al, Ni and Cu, and the 220 reflexions of b.c.c. metals V, Cr and Fe, and the values of f_1^x have been determined by a many-beam calculation with an accuracy as good as that of the most reliable X-ray measurements, and compared with those calculated theoretically for atoms in the solid state (Watanabe, Uyeda & Fukuhara, 1968, 1969; Fujimoto, Terasaki & Watanabe, 1972). The values of E_c for the 400 reflexion of the NaCl type TiO_x crystals ($0.82 \leq x \leq 1.25$) have also been measured and the structure factors for the 200 reflexions have been determined (Watanabe & Terasaki, 1972). In the present study, the values of E_c for the 400 reflexions of f.c.c. metals, Al, Ni and Cu have been measured, and the atomic scattering factors for the 200 reflexions have been determined. The method has also been applied to h.c.p. Ti metal and the atomic scattering factor for the 0002 reflexion has been determined.