

two effects, one ought to succeed in reproducing scattered intensities.*

I am grateful to Dr A. Benedetti for friendly discussions.

* A quantitative analysis of the intensities scattered by some carbon samples is now under way (Ciccariello & Benedetti, 1985b). One compares the goodness of the fits obtained by parameterizing $i(h)$ either according to (1.3) or to Bale & Schmidt (1984), i.e. $Ah^{-\alpha} + B$. (Actually, in the first case, further changes are required if the intensities refer to slit-defined beams.) Our preliminary results, which refer to the intensities reported in Fig. 1 by Perret & Ruland (1968), show no significant differences in the two cases.

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Relative Absorption Correction for Rotation Film Data

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Abstract

An improved method of implementing the absorption correction procedure of Stuart & Walker [*Acta Cryst.* (1979), **A35**, 925–933] is presented. This method scales measured reflections to a partial reference data set of corrected intensities by determining, for each crystal, a transmission surface representing the relative transmission as a function of secondary beam directions. By reformulating the observational equations and by introducing eigenvalue filtering of the least-squares normal equations, the problems of correlation between parameters defining the transmission surface are reduced.

Introduction

Absorption effects can create serious problems in macromolecular crystallography especially with the method of isomorphous replacement, which depends on accurate determination of small intensity differences between reflections recorded from different crystals. A method has been described (Stuart & Walker, 1979; Walker & Stuart, 1983) for dealing with this source of systematic error. The method depends upon

having a reference data set that has been previously corrected for absorption effects and to which the uncorrected measurements are to be scaled, that is

$$I_{\text{ref}}^i = T_{ps} I_{\text{raw}}^i, \quad (1)$$

where I_{ref}^i is the absorption-corrected intensity of the i th reflection, I_{raw}^i is the uncorrected measurement, and T_{ps} is the transmission factor parameterized by the primary and secondary beam directions \mathbf{p} and \mathbf{s} .

In principle, the transmission surface T_{ps} can be determined if a reasonably well distributed set of I_{ref}^i is available. This reference data set ideally consists of data that have been corrected by an empirical method (e.g. Kopfmann & Huber, 1968; North, Phillips & Mathews, 1968), such as partial diffractometer data, which includes a uniformly distributed sample of strong reflections throughout reciprocal space. Alternatively, heavy-atom-derivative data may be scaled to native data, or calculated intensities can be used (Walker & Stuart, 1983).

In this paper we extend the usefulness of the original method and make its application more straightforward. These improvements deal solely with the solution of the least-squares equations and not with any fundamental assumptions of the approach.

The method

To derive the transmission surface T_{ps} from the reference data we use the approximation of Kopfmann & Huber (1968):

$$T_{ps} = T_p T_s, \quad (2)$$

where T_p and T_s are the primary and secondary transmission coefficients respectively. For rotation photographs (following Stuart & Walker, 1979), T_p may be taken as a constant for any single photograph because the movement of the crystal is only a few degrees and T_p is a slowly varying function. It is also convenient to include with T_p the scale factor k_j for the j th film and a pseudo-temperature factor for each film B_j , which describes the relative decrease in intensity of higher-angle reflections caused, for example, by radiation damage. Then we have

$$T_{ps} = k_j \exp(-B_j s^2) T_s. \quad (3)$$

Here the subscript j refers to the j th film pack and $s = \sin\theta/\lambda$. This correction factor will then be used to correct all measured intensity data from the crystal.

The transmission surface for each crystal, T_{ps} , can be expanded as a Fourier series in the spherical angles φ_i , μ_i describing the direction of the secondary beam for the i th reflection in the crystal frame of reference:

$$T_{ps}^{ij} = K_j \exp(-B_j s^2) \left\{ 1 + \sum_m \sum_n [P_{mn} \cos(m\varphi_i + n\mu_i) + Q_{mn} \sin(m\varphi_i + n\mu_i)] \right\}. \quad (4)$$

Here the subscript j refers to the j th film pack and the subscript i refers to the individual reflection; K_j includes k_j and an overall scale on T_s . This expression differs slightly from that of Stuart & Walker (1979) in that the scale factor K_j is factored out, but is related to equation (2.2) of Walker & Stuart (1983), and is similar to the expansion proposed by Katayama, Sakabe & Sakabe (1972) for diffractometer data. Notice that the diffracted rays for different reflections on different films can take similar paths through the crystal yet not have similar positions on their films. Thus this method is different from those where two-dimensional scaling functions are expressed in terms of the positions of reflections on each film (Rossmann, Leslie, Abdel-Meguid & Tsukihara, 1979) and offers a means of combining data from many different films to determine a common transmission surface.

To determine the parameters P and Q defining the part of the transmission surface spanned by the measurements, we minimize by the method of iterative non-linear least squares the expression:

$$\begin{aligned} \Phi^2 &= \sum_i \sum_j w_{ij} (I_{\text{raw}}^i - G_{ij} I_{\text{ref}}^i)^2 \\ &= \sum_i \sum_j w_{ij} (\Delta I)^2, \end{aligned} \quad (5)$$

where $G_{ij} = 1/T_{ps}^{ij}$ and w_{ij} is a weighting factor $1/\sigma_i^2$. Expressing the residuals in terms of the inverse transmission factor renders the weights independent of the parameters being refined (Hamilton, Rollett, & Sparks, 1965).

Two problems arise in the solution of these equations. The first problem pertains to the manner in which a subset of possible parameters $\{P_{mn}, Q_{mn}\}$ is selected for surface fitting. In the algorithm of Stuart & Walker it is necessary to choose a suitable subset of these parameters in advance in order to avoid statistical correlation and its attendant difficulties in solving the least-squares normal equations. Factoring out the scale factor as in (4) above remedies this problem to some extent since otherwise, if left in as the P_{00} term, it is found to be too highly correlated with the first few harmonic terms, a problem alluded to in the example treated by Stuart & Walker. A second problem arises in the case where the diffraction data from one crystal span a relatively small total rotation range so that only part of the transmission surface is sampled. This is often the case with large-unit-cell crystal structures, or with small crystals where radiation damage restricts useful data collection to a small range. In this case, the first few terms of the Fourier expansion (equation 4) become highly correlated because the data fail to sample large enough fractions of their periods. Of course, one of the advantages of a Fourier representation in the case where data cover nearly 360° around the spindle is that the expected symmetry of the transmission surface sets boundary conditions on the solution. With data covering a range less than about 100° the symmetry of the transmission surface around the spindle is not useful in fixing the solution. Indeed, in (4) there is no physical meaning attached to the Fourier coefficients P and Q and the problem should best be thought of in terms of curve fitting or modelling part of the transmission surface. Both of the problems described here lead to ill conditioning of the equations and uncertainties concerning the accuracy of the transmission surface.

Following Diamond (1958), we treat the problem of finding the best set of linearly independent modelling functions by modifying the eigenvalue spectrum of the diagonalized normal equations arising from (5). These orthonormal functions are themselves linear combinations of the Fourier terms appearing in (4) but usually comprise a smaller set. Briefly, if the normal equations are

$$\tilde{E}E\mathbf{x} = \tilde{E}\Delta\mathbf{I}, \quad (6)$$

where \mathbf{x} is the set of parameters (Fourier coefficients, scale factors and shifts in the temperature factors), then, to solve by diagonalization we obtain A the matrix of eigenvectors of $\tilde{E}E$ such that

$$\tilde{A}\tilde{E}EA = \Lambda, \quad (7)$$

where Λ is the diagonal matrix of eigenvalues. The solution to the normal equations, therefore, becomes

$$\mathbf{x} = \Lambda \Lambda^{-1} \tilde{\mathbf{A}} \tilde{\mathbf{E}} \Delta \mathbf{I}. \quad (8)$$

Now, the problems of correlation discussed above can be treated by filtering the matrix Λ^{-1} . By setting to zero those elements of Λ^{-1} greater than some filtering limit, those eigenfunctions of the system that do not contribute strongly to fitting the transmission surface are eliminated.

To alleviate the second problem described above, correlation due to short data range, we modify the period of the fundamental harmonic in (4) in the following manner:

$$\varphi'_i = \varphi_i (2\pi / S \Delta \varphi_{\text{tot}}) \quad (9)$$

$$\mu'_i = \mu_i (2\pi / S \Delta \mu_{\text{tot}}). \quad (10)$$

S is a factor that rescales the period of the Fourier terms. We choose it to be about 2, implying that the first terms of the expansion are half sine or cosine waves. $\Delta \varphi_{\text{tot}}$ and $\Delta \mu_{\text{tot}}$ are the total ranges of secondary beam directions covered by the diffraction data, $\Delta \varphi_{\text{tot}} = \Delta \varphi_{\text{rotation}} + 4\theta_{\text{max}}$, $\Delta \mu_{\text{tot}} = 4\theta_{\text{max}}$.

Discussion

The least-squares equations (5) are of the same form as arise in other methods used to put reflections from different sources on a common scale, such as film-to-film scaling (Hamilton, Rollett & Sparks, 1965; Fox & Holmes, 1966) and post-refinement of crystal orientation from rotation photographs (Winkler, Schutt & Harrison, 1979; Rossmann *et al.*, 1979). Apart from the different parameters used to define a reflection scale factor G_{ij} , these corrections also differ in their sources of reference data I_{ref}^i . For the absorption method described here, and for post-refinement of orientation, the reference data are external to the measured data I_{raw}^i and hence independent of G_{ij} but, for film-to-film scaling, reference intensities are derived from the data themselves, so I_{ref}^i is a function of the G_{ij} . A similar formulation could be used for absorption correction (or indeed all the corrections mentioned could be included simultaneously in the G_{ij}), but this would only give a complete description of the absorption surface if data were included from rotations of one crystal about several different axes.

This method of absorption correction is not restricted to rotation film data. Walker & Stuart (1983) discuss its application to diffractometer data using calculated data as the reference. The method can be easily extended to the continuous rotation geometry used with electronic area detectors. In this case, the film-dependent parameters K_j and B_j can be replaced by functions of the rotation angle.

$$K_j = \sum_i R_i \cos(l\varphi_p) + S_i \sin(l\varphi_p) \quad (11)$$

$$B_j = f(\varphi_p), \quad (12)$$

e.g. a polynomial in φ_p . This would require an external reference data set as described above for film data. Alternatively, Arndt & Thomas (1982) have suggested a strategy for data collection by fast rotation about a number of different axes such that each reflection is measured several times from the same crystal in different geometries. With proper choice of rotation axes, the absorption surface may be extracted from the data, fitting a function of both the primary and secondary beam directions.

The modified method works well with either intensities or amplitudes as the data, and gives well-determined transmission surfaces. Its success depends particularly on the fact that it eliminates correlations between radiation damage effects, as modelled by a temperature factor for each film, and purely absorptive effects, which are a property of the crystal and hence common to all films. Indeed, by using data from all films from one crystal to refine a common transmission surface, a considerable gain in accuracy is achieved over methods where a separate two-dimensional function is determined for each film (discussed by Rossmann *et al.*, 1979). We wish to re-emphasise that finding a transmission surface by this method is essentially a curve-fitting exercise, no physical meaning being attributed to the Fourier coefficients P and Q . It is therefore sensible to adopt an approach whereby a larger number of parameters is initially included in the model leaving for the eigenvalue filter the task of selecting a smaller subset of linear combinations of these parameters for the best curve fitting.

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