A Restrained-Parameter Thermal-Factor Refinement Procedure

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Abstract

Retention of known geometry, with regard to mean atomic positions, has proved useful in the refinement of macromolecules. In structures with a paucity of diffraction data and large displacements of the atoms from their mean positions, it is also of value to restrain the thermal factors to be consistent with known stereochemistry. This paper presents a technique for accomplishing this by restraining the variances of the interatomic distributions (which are functions of the mean atomic positions and the thermal parameters) to suitably small values. This procedure allows meaningful anisotropic refinement of macromolecules to be carried out with low-resolution diffraction data. Anisotropic thermal parameters obtained in this way should prove useful in understanding the dynamics of the biological functions of macromolecules.

Introduction

One objective of single-crystal diffraction experiments is to determine the atomic structure as accurately as possible. This is generally accomplished by first obtaining an approximate atomic model by some means and then refining this model to bring the calculated diffraction pattern into close agreement with the observed pattern. To realize this goal, it is necessary that the atomic model should duplicate the time-average of the electron density present in the unit cells of the crystal. The electron distributions are usually represented by mean atomic positions upon which are superimposed thermal motion and positional disorder distributions. The magnitudes and orientations of these distributions are related to the dynamics of molecular motion. For biologically active macromolecules, these dynamics may, in turn, be related to biological functions of the molecules.

When refining approximate atomic models of macromolecules, it has been useful to retain known geometry during the course of the refinement (Konnert, 1976; Hendrickson & Konnert, 1979). In this way, the number of positional parameters to be determined by the intensity data can be effectively reduced and meaningful refinements can be carried out employing very limited data sets. This retention of known geometry has generally been applied to the mean atomic positions. However, in structures possessing large vibrational amplitudes and other types of positional disorder, it is valuable to consider relationships among thermal factors when restraining a model to be consistent with known geometry. Qualitatively, this means that if an atom undergoes large displacements from its mean position, then certain other atoms related by approximately known stereochemistry must also have related large displacements.

This paper will describe some techniques for restraining individual atom thermal parameters to values consistent with known geometry. In order that restraints might be imposed, it is necessary to relate the individual atom thermal factors which are the experimental variables to the interatomic distance distributions for those distances that are to be restrained. Busing & Levy (1964) discuss various joint distributions corresponding to simplified models for the thermal motion or disorder. Once the form of the joint distribution is assumed, the variance of the interatomic distance distribution is calculated. It is this variance that will be restrained to a suitably small value. A technique that may be employed for restraining such variances, V's, has been described as 'structure-factor least-squares refinement with subsidiary conditions' (Waser, 1963).

The function minimized is of the form

$$\theta = \sum_i w_i (|F_o|_i - |F_c|_i)^2 + \sum_l w_l V_l^2$$

$$+ \text{ sums for other subsidiary conditions,}$$

where V is the variance of the interatomic-distance distribution that is determined by the relative displacements of the two atoms defining interatomic distance l, i may range over all or just a portion of the intensity data, and l ranges over the distances to be restrained. The weight assigned to an observation is w. By associating the w's with standard deviations of the observations, the w's may be chosen such that the V's have the desired distribution.

Formulated in this way, there is a one-to-one correspondence with the restrained parameter refinement previously described for atomic positions (Kon-

nert, 1976). Only the derivative matrix elements related to the restraint information need be calculated and stored to ensure rapid convergence, and the conjugate gradient method for solving linear equations is employed to retain the advantages of the resulting sparse matrix.

This paper will first describe some characteristics of the atomic distributions in macromolecules that indicate that a simplified model for the correlation of atomic motion has sufficient validity to be useful in limiting the number of thermal parameters to be determined by the diffraction data. Next, the general expression for the variance of interatomic-distance distributions will be given as a function of various moments of the assumed joint relative atomic-displacement distribution. Finally, some details and examples will be given for the cases of isotropic and anisotropic thermal parameters.

A preliminary description of this work was presented at the 1978 IUCr Meeting (Konnert & Hendrickson, 1978).

Correlation of atomic motion

For thermal restraints to be rigorously imposed, it would be necessary to know how the atomic motions are correlated. Without such detailed knowledge, some assumptions must be made. In order to discuss what assumptions might be appropriate, it is useful to consider atomic motion along interatomic vectors related to bond distances and bond angles. The root-mean-square (r.m.s.) amplitude for the variation with time of a covalently bonded distance is generally not greater than several hundredths of an ångström. The r.m.s. displacements of atoms from their mean positions in crystals of biological macromolecules are of the order of tenths of ångströms (0·2–0·7 Å). Therefore, the positional disorder, be it vibrational motion or otherwise, of the two atoms along the direction of the covalent bond is highly correlated.

This circumstance may be approximately represented by a joint distribution that assumes a high degree of positive correlation. In one such model, the variances of the joint distributions are expressed as functions of the differences of the variances of the individual atom distributions. Such a motion is termed the 'riding motion' approximation by Busing & Levy (1964). This model, which will be used here, is meant, in this application, to represent only large positive correlation of motion and not rigorous 'riding motion'. In this model, if $\overline{u_A^2}$ is the mean square amplitude of atom A in a given direction and $\overline{u_B^2}$ is the same for atom B, then the mean square amplitude for the relative displacements of the atoms in that direction is $\pm(\overline{u_A^2} - \overline{u_B^2})$, whichever is positive.

Once a form for the joint distribution has been assumed, it is possible to restrain the calculated variances for the covalently bonded distance distributions to suitably small values, usually about $(0·03$ Å$)^2$.

The r.m.s. amplitude for the variation with time of an interatomic distance which, along with two covalently bonded distances, is related to a bond angle is generally of the order of $0·1$ Å, as determined by gas electron-diffraction studies. Since this variation also is smaller than the average total displacement, the assumption of positive correlation is again useful. The variances of such interatomic distance distributions may be restrained to a suitably small value of about $(0·1$ Å$)^2$.

Variance of distance distribution

Let **u** be the instantaneous relative vector displacement of two atoms from their mean positions with a distribution of **u** given by $\rho(\mathbf{u})$. Let the vector separation of the atoms be independent of the position of one of the atoms, B. Then the distribution of the second atom, A, will be represented by $\rho(\mathbf{u})$. Consider a Cartesian coordinate system centered on the mean position of atom A with the x direction along the $A–B$ vector.
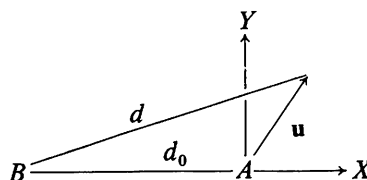
The interatomic distance, d, will then be given by

$$d = [(d_0 + x)^2 + y^2 + z^2]^{1/2},$$

where d_0 is the distance between the mean positions of the atoms. A Maclaurin expansion in x, y and z of d yields

$$d = d_0 + x + y^2/2d_0 + z^2/2d_0 - xy^2/2d_0^2$$
$$- xz^2/2d_0^2 + x^2 y^2/2d_0^3 + x^2 z^2/2d_0^3$$
$$- y^4/8d_0^3 - z^4/8d_0^3 - y^2 z^2/4d_0^3 + \ldots \quad (1)$$

The $\rho(\mathbf{u})$ distributions considered will be symmetric with respect to inversion. In such cases

$$\overline{d^2} = d_0^2 + \overline{x^2} + \overline{y^2} + \overline{z^2} \quad (2)$$

and

$$\bar{d} = d_0 + \overline{y^2}/2d_0 + \overline{z^2}/2d_0 + \overline{x^2 y^2}/2d_0^3$$
$$+ \overline{x^2 z^2}/2d_0^2 - \overline{y^2 z^2}/4d_0^3 - \overline{y^4}/8d_0^3$$
$$- \overline{z^4}/8d_0^3 + \ldots \quad (3)$$

An expression equivalent to (3) is presented by Busing & Levy (1964) as a basis for correcting bond-length estimates for thermal motion. It could be used to restrain $\bar{d}$ instead of d_0 to an 'ideal value'.

The desired variance is then

$$V = \overline{d^2} - (\bar{d})^2 = \overline{x^2} - \overline{y^2\,z^2}/2d_0^2 + \overline{y^2\,z^2}/2d_0^2 - \overline{x^2\,y^2}/d_0^2$$
$$- \overline{x^2\,z^2}/d_0^2 + \overline{y^4}/4d_0^2 + \overline{z^4}/4d_0^2\,(-\overline{y^2})^2/4d_0^2$$
$$- (\overline{z^2})^2/4d_0^2 + \ldots \ldots \quad (4)$$

In order to evaluate V, it is necessary to express $\rho(\mathbf{u})$ as a function of the individual atom thermal parameters.

For the 'riding motion' approximation it is possible to express the moments of $\rho(\mathbf{u})$ in (4) in terms of the moments of the individual atom distributions. This is accomplished in Appendix A. In Appendix B the moments of the individual atom distributions are expressed in terms of the individual atom thermal parameters.

For the 'riding motion' approximation to be physically possible, it is necessary that the distribution for one of the atoms be broader in all directions; i.e. $(\overline{u_A^2} - u_B^2)$ have the same sign for all directions. This condition will always be met when isotropic thermal factors are employed. Extra constraints, however, would be necessary for this condition to be met in general with anisotropic thermal factors. This could be accomplished, and it might be desirable to do so. It has not been done in the work reported here. Instead, a set of variances for one-dimensional approximations corresponding to atomic motion along each of the six principal axes of the two thermal ellipsoids are examined. The variance related to each of these directions is less than or equal to the general 3 D variance. For a case where one ellipsoid is greater in all directions, the near equivalence of the two representations for the variance will be indicated, i.e. the sum of the 1D variances is nearly the same as the 3 D variance.

Variance of distance distribution for one-dimensional atomic motion

Let θ be the angle between the interatomic vector for the mean positions and the direction of interest for relative atomic motion and $\mathbf{u}$ be the coordinate along that direction. The geometry is illustrated below.
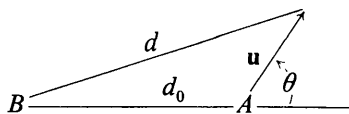
The interatomic distance, d, may be expressed as

$$d = (d_0^2 + 2d_0\,u\cos\theta + u^2)^{1/2}.$$

A Maclaurin series expansion in u of d yields

$$d = d_0 + u\cos\theta + u^2\sin^2\theta/2d_0 - u^3\cos\theta\sin^2\theta/2d_0^2$$
$$+ u^4(4\sin^2\theta\cos^2\theta - \sin^4\theta)/8d_0^3\ldots, \quad (5)$$

with the resulting variance, $[\overline{d^2} - (\bar{d})^2]$, being

$$V = \overline{u^2}\cos^2\theta - (\overline{u^2})^2\sin^4\theta/4d_0^2 + \overline{u^4}\sin^2\theta(\sin^2\theta/4$$
$$- \cos^2\theta)/d_0^2. \quad (6)$$

Comparison of a 3 D distance variance with the sum of 1 D variances

As stated previously, the variance for the general three-dimensional distribution was not restrained in the anisotropic refinement to be discussed. In order to obtain some insight into how the 1 D restraints that were used might be related to a general 3 D treatment, it is instructive to look at what (4) reduces to for the special case when the thermal ellipsoids for atoms A and B have axes coincident with the coordinate system of (4). The relative motion distribution, $\rho(\mathbf{u})$, is then also Gaussian. Referring to Appendices A and B to evaluate the moments in (4), one obtains

$$V = \Delta_x^2 - \Delta_x^2\,\Delta_y^2/d_0^2 - \Delta_x^2\,\Delta_z^2/d_0^2 + \Delta_y^4/2d_0^2$$
$$+ \Delta_z^4/2d_0^2 + \ldots \ldots \quad (7)$$

The difference in mean square amplitudes along the principal axes is denoted by $\Delta_u^2 = (\overline{u_A^2} - u_B^2)$, where $u = x, y,$ or z.

This may be compared with a sum of the 1 D V's given by (6) for the case of Gaussian motion, whereby

$$V_u = \Delta_u^2\cos^2\theta + \Delta_u^4\sin^4\theta/2d_0^2$$
$$- 3\Delta_u^4\cos^2\theta\sin^2\theta/d_0^2\ldots\ldots \quad (8)$$

Evaluation at $\theta = 0$, 90 and 90° for motion along x, y and z, respectively, gives

$$V_x + V_y + V_z = \Delta_x^2 + \Delta_y^4/2d_0^2 + \Delta_z^4/2d_0^2. \quad (9)$$

The cross terms by which (7) and (9) differ are not important in the usual circumstances. If all Δ's are of about the same size, then the first term in (7) will be dominant and the last four terms will be negligible. If Δ_x is relatively small so that the first term is not dominant, then the cross terms will be small compared with one or both of the last two terms. This indicates that, for this special case, the variance estimate from the sum of three orthogonal 1 D variances is quite similar to that obtained from the general 3 D expression. It should be emphasized again, however, that for a general 3 D treatment of 'riding motion', one atom must have greater thermal motion or disorder than the other atom in all directions. When this is not the case, an expression such as (7) cannot be written.

Isotropic thermal-factor restraint

For the case with isotropic thermal factors, the expression for V given in (4) simplifies with the relationships given in Appendices A and B to

$$V = \Delta^2 - \Delta^4/d_0^2 + \ldots \simeq \Delta^2, \quad (10)$$

where $\Delta^2 = \pm(u_A^2 - u_B^2) = \pm(B_A - B_B)/8\pi^2$, whichever is positive. The derivatives necessary for the Taylor expansion of the variance in the least-squares calculation are of the form

$$\partial V/\partial B_A = \pm 1/8\pi^2. \qquad (11)$$

Isotropic applications

Isotropic thermal-factor restraints have been incorporated into exported programs for stereochemically restrained refinement of macromolecules (Hendrickson & Konnert, 1979) and are now being used routinely in the refinements of a number of protein structures. One of the first applications to include these thermal-factor restraints was in the refinement of yeast phenylalanyl-tRNA. This structure including 1710 atoms has been refined to a conventional R of 21·5% for the 10 195 reflections between 10 and 2·5 Å. Positional restraints held the r.m.s. deviation from bond ideality to 0·04 Å. The r.m.s. variance from thermal-factor restraints (10) ranged from $(0·05$ Å$)^2$ for bond distances in the nucleotide bases to $(0·11$ Å$)^2$ for bonding angle distances in the ribose–phosphate backbone. Despite this close conformity of thermal factors to known stereochemistry, large and meaningful variation in B values is exhibited throughout the molecule as is illustrated in Fig. 1. The inclusion of this thermal-factor treatment in the refinement also greatly improved the clarity of difference maps and permitted the identification of bound


Fig. 1. A drawing of yeast phenylalanyl-tRNA representing the backbone isotropic thermal factors for different regions of the molecule. The region with $25 < B$ are represented with white; those with $20 < B < 25$, with the lightest shading; those with $15 < B < 20$, with the medium shading; and those with $B < 15$, with the darkest.

spermine molecules and magnesium hydrate ions (Quigley, Teeter & Rich, 1978).

Anisotropic thermal-factor restraints

For the refinement illustrated here, the principal axes directions of the ellipsoids have been specified by the atomic environment of each atom in order to reduce the number of parameters to be determined by the diffraction data. The directions were chosen so as to coincide as nearly as possible with expected directions of maximum and minimum displacements. For example, amplitudes are often greater perpendicular to bonds and the planes of planer groups than along covalent-bond directions. Consider an amide group:

$$
\begin{array}{c}
3 \\
\uparrow \\
O \rightarrow 1 \\
\| \\
C \\
/ \;\; \backslash \\
N \quad C_\alpha
\end{array}
$$
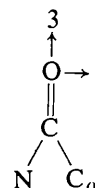
Axis 1 of the oxygen atom may be chosen parallel to the N—C_α direction and axis 2 may be set by the cross product of the N—C and C—O directions. The vibrational parameter describing motion in the direction of axis 2, i.e. a direction perpendicular to the plane of the amide group, might be expected to assume a relatively large value during the course of the refinement.

There will be three refinable thermal parameters per atom. However, due to the restraints to be placed upon them, the effective number of such parameters to be determined by the diffraction data will be considerably less. This reduction in parameters is most important when investigating macromolecules in which the atoms are undergoing large excursions from their mean positions and, as a result, the diffraction data are limited.

Choosing inaccurate axes directions should result primarily in the ellipsoids for that atom being more nearly isotropic than might best fit the data. In fact, quite accurate axes assignments may be made from the considerations mentioned. This has been indicated by the refinement in this laboratory of an unsymmetric trimer, $C_{60}H_{42}N_4Cl_3$ both with stereochemical restraints and with conventional full-matrix least-squares (Konnert, Flippen-Anderson and Gilardi, 1979). The restrained anisotropic refinement of the 109 atoms converged in five cycles from an R of 28 to 7·2% for the 6000 data with $\sin \theta/\lambda < 0·55$. Heavy-atom positions from an E map, idealized hydrogen-atom positions, and equal thermal values provided the starting point. The full-matrix refinement of the 67 non-hydrogen atoms converged to 7·6% with the hydrogens placed in idealized positions with the same thermal

factors as the heavier atoms to which they were attached. Positional and thermal parameters obtained by the two methods are very similar.

When the thermal factor for an atom is of the form $\exp(-TFE)$, TFE may be expressed in the following form for an anisotropic thermal ellipsoid.

$$TFE = \beta_1(\varepsilon_1 . \mathbf{h}')^2 + \beta_2(\varepsilon_2 . \mathbf{h}')^2 + \beta_3(\varepsilon_3 . \mathbf{h}')^2. \quad (12)$$

In this expression, $\mathbf{h}'$ is the reciprocal-lattice vector

$$\mathbf{h} = \begin{pmatrix} ha^* \\ kb^* \\ lc^* \end{pmatrix}$$

transformed with the matrix G to a Cartesian coordinate system:

$$\mathbf{h}' = G\mathbf{h}.$$

The unit vector in the direction of β_i in the same system is ε_i. $\beta_i = 2\pi^2 \overline{u_i^2}$, where $\overline{u_i^2}$ is the mean square amplitude along that axis.

Appendices A and B can be used to simplify the variance of the distance distribution from that given in (6) to

$$V_v = \Delta_v^2 \cos^2 \theta + \Delta_v^4 (\tfrac{1}{2} \sin^4 \theta - 3 \cos^2 \theta \sin^2 \theta)/d_0^2, \quad (13)$$

where

$$\Delta_v^2 = \pm (\overline{u_{v,A}^2} - \overline{u_{v,B}^2}), \quad (14)$$

whichever is positive. The values of the mean square displacements of the individual atoms in an arbitrary direction specified by $\mathbf{v}$, a unit vector in the Cartesian system, will be necessary for the calculation of the variance in that direction. These are given by

$$2\pi^2 \overline{u_v^2} = \beta_1(\mathbf{v}.\varepsilon_1)^2 + \beta_2(\mathbf{v}.\varepsilon_2)^2 + \beta_3(\mathbf{v}.\varepsilon_3)^2. \quad (15)$$

The desired derivatives to be used in the least-squares procedure are of the form:

$$\frac{\partial V}{\partial \beta_{i,A}} = \pm \frac{(\varepsilon_{i,A} . \mathbf{v})^2}{2\pi^2} [\cos^2 \theta + \frac{2\Delta^2}{d_0}(\tfrac{1}{2} \sin^4 \theta$$
$$- 3 \cos^2 \theta \sin^2 \theta)]; \quad (16)$$

$$\frac{\partial V}{\partial \beta_{j,B}} = \mp \frac{(\varepsilon_{j,B} . \mathbf{v})^2}{2\pi^2} [\cos^2 \theta + \frac{2\Delta^2}{d_0}(\tfrac{1}{2} \sin^4 \theta$$
$$- 3 \cos^2 \theta \sin^2 \theta)].$$

The upper sign on the right hand side of these equations holds if $u_A^2 > u_B^2$, where A and B correspond to the atoms involved in the distance of interest.

It is evident that the same general procedure could be employed without specifying the ellipsoid orientations. There would then be the usual six thermal parameters per atom.

The stereochemical restraints on the mean atomic positions might be used as a basis for a more formal method for determining the initial orientations of the ellipsoids. Briefly stated, this might be accomplished with a restrained-parameter least-squares technique in which a directional force is applied to the atoms in the molecule. The atomic displacements permitted by the known stereochemistry for such a force may be calculated. By applying a distorting force in six different directions and obtaining the related atomic shifts, it should be possible to approximate the parameters for the associated constant-probability ellipsoids. The orientations of these ellipsoids could be employed to initiate refinement.

It is also possible to examine the relative amplitudes of atoms in approximately rigid groups (Schomaker & Trueblood, 1968), restrain the thermal parameters to be consistent with group motion, and make improved axes assignments during the course of the refinement. This is accomplished by fitting at the beginning of each refinement cycle the thermal factors that define a group motion to the anisotropic thermal factors for the atoms of an approximately rigid group. The β_i's in (12) may then be restrained with a set of subsidiary conditions to remain consistent to within desired limits with the obtained group motion parameters. The ellipsoid axes directions for the atoms in this group may also be reassigned to be consistent with those for group motion. This is now being done in this laboratory.

Anisotropic application

Data from a carp muscle parvalbumin, a calcium-binding protein that has been previously refined by other techniques (Moews & Kretsinger, 1975), have been employed for test calculations. With the inclusion of 152 solvent atoms, the 2892 positional parameters
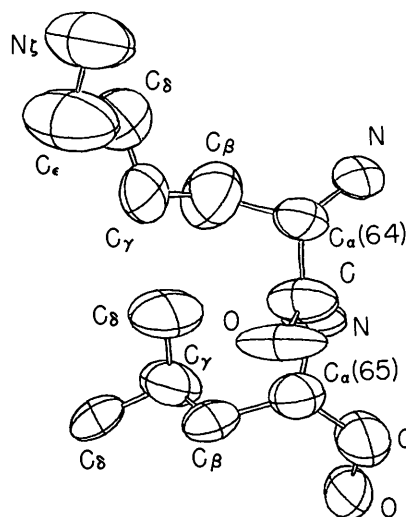

Fig. 2. An *ORTEP* drawing (Johnson, 1965) at the 50% probability level for 2% of the carp calcium-binding parvalbumin molecule at $R = 9.8\%$.

and the 2892 thermal parameters for the 952 atoms were simultaneously refined. 5100 diffraction data with d spacings between 5 and 1·8 Å were employed. The structure was refined to a conventional R value of 9·8%. A portion of the structure is illustrated in Fig. 2. The average value for the variances (equation 7) was $(0·03 Å)^2$ at this stage. Visual inspection of the constant-probability ellipsoids in Fig. 2 reveals the near equivalence for the ellipsoids of bonded atoms along the bond direction. Likewise, the ellipsoids of next-nearest neighbor atoms are very similar along the interatomic vector.

APPENDIX A

Evaluation of the moments of the relative displacement distribution for the 'riding motion' approximation

The approach employed here is very similar to that employed by Busing & Levy (1964). Given two normalized distributions, $\rho(r)$ for the distribution of the relative displacements of atom A and atom B and $\rho_B(r)$ for the distribution of atom B, each with centroid at $r = 0$, their convolution is

$$\rho_A(r) = \int \rho_B(s)\,\rho(r - s)\,ds,$$

which gives the distribution of atom A when it is 'riding' along with atom B. Let x be given by $x = r.k$, where k is a unit vector. The value for $\overline{x_A^2}$ is obtained by averaging over the convolution

$$\overline{x^2} = \int (r.k)^2 \rho_A(r)\,dr$$

$$= \int (r.k)^2 \int \rho_B(s)\,\rho(r-s)\,ds\,dr.$$

It is now convenient to change variables such that $u = r - s$ and $r = u + s$. The relevant Jacobian for the change of variables is

$$J = \begin{vmatrix} \partial s/\partial s & \partial s/\partial u \\ \partial r/\partial s & \partial r/\partial u \end{vmatrix} = \begin{vmatrix} 1 & 0 \\ 1 & 1 \end{vmatrix} = 1.$$

Therefore,

$$\overline{x_A^2} = \int \rho(u) \int (s.k + u.k)^2 \rho_B(s)\,ds\,du$$

$$= \int \rho(u) \int [(s.k)^2 + 2(s.k)(u.k)$$

$$+ (u.k)^2] \rho_B(s)\,ds\,du$$

$$= \overline{x^2} + 2\overline{x}\,\overline{x_B} + \overline{x_B^2} = \overline{x^2} + \overline{x_B^2}$$

and

$$\overline{x^2} = \overline{x_A^2} - \overline{x_B^2}.$$

Next, $\overline{x_A^2 y_A^2}$ will be determined. Let $y = 1.r$, and proceeding as before:

$$\overline{x_A^2 y_A^2} = \int \rho(u) \int (u.k + s.k)^2 (u.l + s.l)^2 \rho_B(s)\,ds\,du$$

$$= \int \rho(u) \int [(u.k)^2 (u.l)^2 + 2(u.k)^2 (u.l)(s.l)$$

$$+ (u.k)^2 (s.l)^2 + 2(u.k)(s.k)(u.l)^2$$

$$+ 4(u.k)(s.k)(u.l)(s.l) + 2(u.k)(s.k)(s.l)^2$$

$$+ (s.k)^2 (u.l)^2 + 2(s.k)^2 (u.l)(s.l)$$

$$+ (s.k)^2 (s.l)^2] \rho_B(s)\,ds\,du$$

$$= \overline{x^2 y^2} + \overline{x^2 y_B^2} + \overline{x_B^2 y^2} + \overline{x_B^2 y_B^2}.$$

Therefore,

$$\overline{x^2 y^2} = \overline{x_A^2 y_A^2} - (\overline{x_A^2} - \overline{x_B^2})\overline{y_B^2} - (\overline{y_A^2} - \overline{y_B^2})\overline{x_B^2} - \overline{x_B^2 y_B^2}.$$

A fourth moment, $\overline{y_A^4}$, will be determined in the same manner:

$$\overline{y_A^4} = \int \rho(u) \int (u.l + s.l)^4 \rho_B(s)\,ds\,du$$

$$= \int \rho(u) \int [(u.l)^4 + 4(u.l)^3 (s.l)$$

$$+ 6(u.l)^2 (s.l)^2 + 4(u.l)(s.l)^3$$

$$+ (s.l)^4]ds\,du$$

$$= \overline{y^4} + 6\overline{y^2}\,\overline{y_B^2} + \overline{y_B^4}.$$

This gives

$$\overline{y^4} = \overline{y_A^4} - 6(\overline{y_A^2} - \overline{y_B^2})\overline{y_B^2} - \overline{y_B^4}.$$

Thus, all of the moments in (4) may be expressed in terms of the moments of the individual atom distributions. Note, again, that for all of the above moments to have physical significance, the distribution of atom A must be broader in all directions than that for atom B.

The expression for the variance in terms of the moments of the individual atom distributions is

$$V = \overline{x_A^2} - \overline{x_B^2} + \frac{1}{2d_0^2} [\overline{y_A^2 z_A^2} - \overline{y_A^2 z_A^2} + \overline{y_B^2 z_B^2} - \overline{z_B^2 y_B^2}]$$

$$- \frac{1}{d_0^2} \overline{x_A^2 y_A^2} - (\overline{x_A^2} - \overline{x_B^2})\overline{y_B^2} - (\overline{y_A^2} - \overline{y_B^2})\overline{x_B^2} - \overline{x_B^2 y_B^2}]$$

$$- \frac{1}{d_0^2} [\overline{x_A^2 z_A^2} - (\overline{x_A^2} - \overline{x_B^2})\overline{z_B^2} - (\overline{z_A^2} - \overline{z_B^2})\overline{x_B^2} - \overline{x_B^2 z_B^2}]$$

$$+ \frac{1}{4d_0^2} [\overline{y_A^4} - (\overline{y_A^2})^2 + 5(\overline{y_B^2})^2 - \overline{y_B^4} - 4\overline{y_A^2}\,\overline{y_B^2}]$$

$$+ \frac{1}{4d_0^2} [\overline{z_A^4} - (\overline{z_B^2})^2 + 5(\overline{z_B^2})^2 - \overline{z_B^4} - 4\overline{z_A^2}\,\overline{z_B^2}].$$

APPENDIX B

Evaluation of the moments of the individual atom distributions in terms of the thermal-factor parameters

Let uvw be the reference Cartesian system for the anisotropic representation, $\varphi(u, v, w)$, for the atom displacements of an atom. Then,

$$\varphi_{(u,v,w)} = [1/(2\pi)^{3/2}\, \sigma_u\, \sigma_v\, \sigma_w]\, \exp\,(-u^2/2\sigma_u^2 - v^2/2\sigma_v^2 - w^2/2\sigma_w^2).$$

Let T_{xu}, T_{xv} and T_{xw} be the direction cosines of a direction x relative to u, v and w for atom A. Then, $x = uT_{xu} + vT_{xv} + wT_{xw}$ and

$$\overline{x_A^2} = \int (uT_{xu} + vT_{xv} + wT_{xw})^2\, \varphi(u, v, w)\, du\, dv\, dw$$

$$= \sigma_u^2\, T_{xu}^2 + \sigma_v^2\, T_{xv}^2 + \sigma_w^2\, T_{xw}^2.$$

These σ's are directly related to the anisotropic thermal factors given in (15) that are the refined parameters

$$\sigma_u^2 \times 2\pi^2 = \beta_u.$$

A fourth moment may be evaluated:

$$\overline{x_A^4} = 3(\sigma_u^4\, T_{xu}^4 + \sigma_v^4\, T_{xv}^4 + \sigma_w^4\, T_{xw}^4) + 6(\sigma_u^2 \sigma_v^2 T_{xu}^2 T_{xv}^2$$
$$+ \sigma_u^2 \sigma_w^2 T_{xu}^2 T_{uw}^2 + \sigma_v^2 \sigma_w^2 T_{xv}^2 T_{xw}^2).$$

In the same manner, the mixed moments may be evaluated:

$$\overline{x_A^2 y_A^2} = 3(\sigma_u^4\, T_{xu}^2\, T_{yu}^2 + \sigma_v^4\, T_{xv}^2\, T_{yv}^2 + \sigma_w^4\, T_{xw}^2\, T_{yw}^2)$$

$$+ \sigma_u^2 \sigma_v^2 (T_{xu}^2\, T_{yv}^2 + T_{xv}^2\, T_{yu}^2$$
$$+ 4T_{xu}\, T_{yv}\, T_{xv}\, T_{yu}) + \sigma_u^2 \sigma_w^2 (T_{xu}^2\, T_{yw}^2$$
$$+ T_{xw}^2\, T_{yu}^2 + 4T_{xu}\, T_{yw}\, T_{yu}\, T_{xu})$$
$$+ \sigma_v^2 \sigma_w^2 (T_{xv}^2\, T_{yw}^2 + T_{xw}^2\, T_{yv}^2$$
$$+ 4T_{xv}\, T_{yw}\, T_{xw}\, T_{yv}).$$

With these relationships, the variance given in (4) is related to the anisotropic thermal parameters being refined.

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Symmetry Determination of the Room-Temperature Form of LnNbO4 (Ln = La,Nd) by Convergent-Beam Electron Diffraction

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Abstract

Three different space groups, $C2/c$, $C2$ and Cc, have been reported for the room-temperature form of the rare-earth orthoniobates, $LnNbO_4$ (Ln = La,Nd). They belong to different point groups: $2/m$, 2 and m, respectively. By means of convergent-beam electron diffraction, the point group has been determined to be $2/m$ in the present study. Therefore the true space group must be $C2/c$.

It has been reported that the rare-earth orthoniobates, $LnNbO_4$ (Ln = La,Nd), transform from the high-temperature tetragonal form with space group $I4_1/a$ to the room-temperature monoclinic form with space group $C2$ (Komkov, 1959; Stubican, 1964). Recently, however, Jeitschko, Sleight, McClellan & Weiher (1976) suggested that the point group of the room-temperature form is centrosymmetric, $2/m$, and Brixner, Whitney, Zumsteg & Jones (1977) reported that they transform from $I4_1/a$ to $C2/c$. On the other hand, Tsunekawa, Takei & Ishigame (1977) proposed from a