matched to a Buckingham potential (Campbell Smith & Arnott, 1978). A variety of other restraints can also be incorporated.

In the *LALS* system, the quantity Ω given by

$$\Omega = \sum_m \omega_m \Delta F_m^2 + \sum_m k_m \Delta d_m^2 + \sum_m \lambda_m G_m = X + C + L \quad (4.5.2.62)$$

is minimized by varying a set of chosen parameters consisting of conformation angles, possibly bond angles, and packing parameters. The term X involves the differences ΔF_m between the model and experimental X-ray amplitudes – Bragg and/or continuous. The term C involves restraints to ensure that overshort nonbonded interatomic distances are driven beyond acceptable minimum values, that conformations are within desired domains, that hydrogen-bond and coordination geometries are close to the expected configurations, and a variety of other relationships are satisfied (Campbell Smith & Arnott, 1978). The ω_m and k_m are weights that are inversely proportional to the estimated variances of the data. The term L involves constraints which are relationships that are to be satisfied exactly $(G_m = 0)$ and the λ_m are Lagrange multipliers. Constraints are used, for example, to ensure connectivity from one helix pitch to the next and to ensure that chemical ring systems are closed. The cost function Ω is minimized using full-matrix nonlinear least squares and singular value decomposition (Campbell Smith & Arnott, 1978).

Structure determination usually involves first using equation (4.5.2.62) with the terms C and L only, to establish the stereochemical viability of each kind of possible molecular model and packing arrangement. It is worth emphasizing that it is usually advantageous if the specimen is polycrystalline, even though the continuous diffraction contains, in principle, more information than the Bragg reflections (since the latter are sampled). This is because the molecule in a noncrystalline specimen must be refined in steric isolation, whereas for a polycrystalline specimen it is refined while packed in the crystal lattice. The extra information provided by the intermolecular contacts can often help to eliminate incorrect models. This can be particularly significant if the molecule has flexible sidechains. The initial models that survive the steric optimization are then optimized also against the X-ray data, by further refinement with X included in equation (4.5.2.62). The ratios $(\Omega_P/\Omega_Q)^{1/2}$ and $(X_P/X_Q)^{1/2}$ can be used in Hamilton's test (Hamilton, 1965) to evaluate the differences between models P and Q. On the basis of these statistical tests, one can decide if one model is superior to the others at an acceptable confidence level. In the final stages of refinement, bond angles may be varied in a 'stiffly elastic' fashion from their mean values if there are sufficient data to justify the increase in the number of degrees of freedom.

If sufficient X-ray data are available, it is sometimes possible to locate additional ordered molecules such as counterions or solvent molecules by difference Fourier synthesis as described in Section 4.5.2.6.5. Their positions can then be co-refined with the polymer structure while hydrogen bonds and coordination geometries are optimized. The resulting structure can then be used to compute improved phases to search for additional molecules. Since the signal-to-noise ratio in fibre difference syntheses is usually low, difference maps must be interpreted with caution. The assignment of counterions or solvent molecules to peaks in the difference synthesis must be supported by plausible interactions with the rest of the structure and, following refinement of the structure, by elimination of the peak in the difference map and by a significant improvement in the agreement between the calculated and measured X-ray amplitudes.

4.5.2.6.5. *Difference Fourier synthesis*

Difference Fourier syntheses are widely used in both protein and small-molecule crystallography to detect structural errors or to complete partial structures (Drenth, 1994). The difficulty in applying difference Fourier techniques in fibre diffraction is that the individual observed amplitudes $|F_o|$ are not available. However, difference syntheses have found wide use in fibre diffraction analysis, one of the earliest applications being to polycrystalline fibres of polynucleotides (*e.g.* Arnott *et al.*, 1967). Calculation of a three-dimensional difference map (for the unit cell) from Bragg fibre diffraction data requires that the observed intensity $I_I(R_{hk}) = I_o$ be apportioned among the contributing intensities $|F_{hkl}|^2 = |F_o|^2$. There are two ways of doing this. The intensities may be divided equally among the contributing $(m/2)$ reflections [*i.e.* $|F_o| = (2I_o/m)^{1/2}$], or they may be divided in the same proportions as those in the model, *i.e.*

$$|F_o| = \left(\frac{I_o}{\sum |F_c|^2}\right)^{1/2} |F_c|. \quad (4.5.2.63)$$

The advantage of the former is that it is unbiased, and the advantage of the latter is that it may be more accurate but is biased towards the model. Equal division of the intensities is often (but not always) used to minimize model bias. Once the observed amplitudes have been apportioned, an $|F_o| - |F_c|$ map can be calculated as in conventional crystallography, although noise levels will be higher owing to errors in apportioning the amplitudes. As a result of overlapping of the reflections, a synthesis based on coefficients $m|F_o| - (m-1)|F_c|$ gives a more accurate estimate of the true density than does one based on $2|F_o| - |F_c|$, as is described below. Difference syntheses for polycrystalline specimens calculated in this way have been used, for example, to locate cations and water molecules in polynucleotide and polysaccharide structures (*e.g.* Cael *et al.*, 1978), to help position molecules in the unit cell (*e.g.* Chandrasekaran *et al.*, 1994) and to help position side chains, and have also been applied in neutron fibre diffraction studies of polynucleotides (Forsyth *et al.*, 1989).

Sim (1960) has shown that the mean-squared error in difference syntheses can be minimized by weighting the coefficients based on the agreement between the calculated and observed structure amplitudes. Such an analysis has recently been conducted for fibre diffraction, and shows that the optimum difference synthesis is obtained by using coefficients (Millane & Baskaran, 1997; Baskaran & Millane, 1999*a*)

$$\left[w_m \frac{|F_c|(I_o)^{1/2}}{(\sum |F_c|^2)^{1/2}} - |F_c|\right] \exp(i\alpha_c), \quad (4.5.2.64)$$

where m is the number of degrees of freedom as defined in Section 4.5.2.6.1. If the reflections contributing to I_o are either all centric or all acentric, then the weights are given by

$$w_m = \frac{I_{m/2}(X)}{I_{m/2-1}(X)}, \quad (4.5.2.65)$$

where $I_m(\cdot)$ denotes the modified Bessel function of the first kind of order m, and X is given by

$$X = \frac{\kappa (I_o)^{1/2} (\sum |F_c|^2)^{1/2}}{\sum_j f_j^2}, \quad (4.5.2.66)$$

where $\kappa = 1$ for centric reflections and 2 for acentric reflections. The form of the weighting function is more complicated if both centric and acentric reflections contribute, but it can be approximated as w' given by

$$w' = (w_{2N_a} + w_{N_c})/2, \quad (4.5.2.67)$$

where N_a and N_c are the number of acentric and centric reflections,

respectively, contributing. Use of the weighted maps reduces bias towards the model (Baskaran & Millane, 1999*b*).

For continuous diffraction data from noncrystalline specimens, the situation is essentially identical except that one works in cylindrical coordinates. Referring to equations (4.5.2.7) and (4.5.2.10), the desired difference synthesis, $\Delta g(r,\varphi,z)$, is the Fourier–Bessel transform of $G_o - G_c$ where G_o and G_c denote the observed and calculated, respectively, Fourier–Bessel structure factors $G_{nl}(R)$. Since G_o is not known, the synthesis is based on the Fourier–Bessel transform of $(|G_o| - |G_c|)\exp(i\alpha_c)$, where α_c is the phase of G_c. As in the polycrystalline case, the individual $|G_o|$ need to be estimated from the data $I_o^{1/2}$ given by equation (4.5.2.17), and can be based on either equal division of the data, or division in the same proportion as the amplitudes from the model.

Namba & Stubbs (1987*a*) have shown that the peak heights in a difference synthesis are $1/m$ times their true value, as opposed to half their true value in a conventional difference synthesis. The best estimate of the true map is therefore provided by a synthesis based on the coefficients $[m|F_o| - (m-1)|F_c|]\exp(i\alpha_c)$, rather than on $(2|F_o| - |F_c|)\exp(i\alpha_c)$. Test examples showed that the noise in the synthesis can be reduced by using a value for m that is fixed over the diffraction pattern and approximately equal to the average value of m over the pattern (Namba & Stubbs, 1987*a*). Difference Fourier maps for noncrystalline systems have been used in studies of helical viruses to locate heavy atoms, to correct errors in atomic models and to locate water molecules (Mandelkow *et al.*, 1981; Lobert *et al.*, 1987; Namba, Pattanayek & Stubbs, 1989; Wang & Stubbs, 1994).

4.5.2.6.6. *Multidimensional isomorphous replacement*

At low enough resolution, only one Fourier–Bessel structure factor contributes on each layer line of a fibre diffraction pattern, so that only the phase needs to be determined and the situation is no different to that in protein crystallography. If heavy-atom-derivative specimens can be prepared, the usual method of multiple isomorphous replacement (MIR) (Drenth, 1994) can be applied, which in principle requires only two heavy-atom derivatives. At higher resolution, however, more than one Fourier–Bessel structure factor contributes on each layer line. A generalized form of isomorphous replacement which involves using diffraction data from several heavy-atom derivatives to determine the real and imaginary components of each contributing $G_{nl}(R)$ is referred to as *multidimensional isomorphous replacement* (MDIR) (Namba & Stubbs, 1985). MDIR was first described and used to determine the structure of TMV at 6.7 Å resolution (Stubbs & Diamond, 1975; Holmes *et al.*, 1975), and has since been used to extend the resolution to 2.9 Å (Namba, Pattanayek & Stubbs, 1989). A consequence of cylindrical averaging is that large numbers of heavy-atom derivatives are required: at least two for each Bessel term to be separated. The theory of MDIR is outlined here.

The first step in MDIR is location of the heavy atoms in the derivative structures. The radial coordinate of a heavy atom can be determined by analysis of the intensity distribution in the low-resolution region of the equator where only the $G_{00}(R)$ Bessel term contributes. Since $G_{00}(R)$ is real, and $I_l(R)$ can be measured continuously in R, inspection of the positions of the minima and maxima in the low-resolution region of the equator generally allows the sign of $G_{00}(R)$ to be assigned to $I_0^{1/2}(R)$, *i.e.* $G_{00}(R)$ can be determined from $I_0(R)$. If the sign is determined for both the native and a heavy-atom derivative, referring to equation (4.5.2.13) shows that

$$G_{00}^D(R) - G_{00}(R) = o_h f_h J_0(2\pi R r_h), \tag{4.5.2.68}$$

where $G_{00}^D(R)$ is the value derived from the derivative data, o denotes the occupancy and the subscript h denotes values for the heavy atom. The parameters o_h and r_h on the right-hand side of equation (4.5.2.68) can be searched in a trial-and-error fashion to obtain the best agreement with the left-hand side (calculated from the data) to determine the radial coordinate r_h of the heavy atom (Mandelkow & Holmes, 1974). Lobert *et al.* (1987) applied the same method to cucumber green mottle mosaic virus (CGMMV), except that the sign of $G_{00}(R)$ was taken from that of TMV.

Two approaches have been used to determine the angular and axial coordinates of the heavy atom. Mandelkow & Holmes (1974) and Holmes *et al.* (1975) used a search procedure in which the quantity $\Phi = -n\varphi_h + 2\pi l z_h/c$ is varied and used to calculate the intensity of the Fourier–Bessel structure factor for the heavy atom alone. This is compared to $I_l^D(R) - I_l(R)$ on each layer line, where only one Bessel order contributes, and Φ chosen to minimize the mean-square difference. The values of Φ found for each layer line can then be combined to determine φ_h and z_h. In the case of CGMMV, Lobert *et al.* (1987) used the phases and Bessel-order separations from TMV to calculate Fourier–Bessel difference maps between the native and derivative data to determine the heavy-atom coordinates (r_h, φ_h, z_h).

Consider a set of isomorphous heavy-atom derivatives indexed by j. Since the analysis is applied at any point (l,R) on the fibre diffraction pattern, the symbol G_n will be used for $G_{nl}(R)$ where no confusion arises. Denote by $G_{n,j}$ the value of G_n for the jth derivative, so that

$$G_{n,j} = G_n + g_{n,j}, \tag{4.5.2.69}$$

where $g_{n,j}$ denotes the Fourier–Bessel structure factor of a structure containing the heavy atom only. Denote by A_n and B_n the real and imaginary parts, respectively, of G_n (for the native structure), and by $a_{n,j}$ and $b_{n,j}$ the real and imaginary parts of $g_{n,j}$, *i.e.* for the jth heavy-atom structure alone. Equation (4.5.2.17) can then be written as

$$I = \sum_n (A_n^2 + B_n^2) \tag{4.5.2.70}$$

for the native and

$$I_j = \sum_n [(A_n + a_{n,j})^2 + (B_n + b_{n,j})^2] \tag{4.5.2.71}$$

for the jth derivative. If intensity data are available from J heavy-atom derivatives, $a_{n,j}$ and $b_{n,j}$ can be calculated from the heavy-atom positions, and equations (4.5.2.70) and (4.5.2.71) represent a system of $J+1$ second-order equations for the m unknowns A_n and B_n. If $J+1 > m$, then the system of equations is overdetermined and can be solved for the A_n and B_n. The solution of this nonlinear system can be eased by deriving a system of linear equations by substituting from (4.5.2.70) into (4.5.2.71), giving

$$\sum_n (A_n a_{n,j} + B_n b_{n,j}) = (1/2)\left[I_j - I - \sum_n (a_{n,j}^2 + b_{n,j}^2)\right]. \tag{4.5.2.72}$$

Equation (4.5.2.72) is a system of linear equations for the unknowns A_n and B_n, the solution being subject to the constraint equation (4.5.2.70). However, since the original problem is second-order, there may be up to m local minima. Stubbs & Diamond (1975) describe a numerical procedure for locating *all* the local minima and selecting the best of these based on 'continuity' of the $G_{nl}(R)$. This method was used to determine the structure of TMV at 6.7 Å resolution (Holmes *et al.*, 1975) and 4 Å resolution (Stubbs *et al.*, 1977). In current applications of MDIR a more direct solution technique is used in which the phase-determining equations (4.5.2.70) and (4.5.2.71) are solved by first solving the linear equations (4.5.2.72) by linear least squares to obtain an approximate