$$E^2 = \sum_n (F_{HLE} - F_H)^2/n. \qquad (2.4.4.32)$$

2.4.4.7. *Suggested modifications to Blow and Crick formulation and the inclusion of phase information from other sources*

Modifications to the Blow and Crick procedure of phase evaluation have been suggested by several workers, although none represent a fundamental departure from the essential features of their formulation. In one of the modifications (Cullis *et al.*, 1961*a*; Ashida, 1976), all E_i's are assumed to be the same, but the lack-of-closure error ξ_{Hi} for the ith derivative is measured as the distance from the mean of all intersections between phase circles to the point of intersection of the phase circle of that derivative with the phase circle of the native protein. Alternatively, individual values of E_i are retained, but the lack of closure is measured from the weighted mean of all intersections (Ashida, 1976). This is obviously designed to undo the effects of the unduly high weight given to F_N in the Blow and Crick formulation. In another modification (Raiz & Andreeva, 1970; Einstein, 1977), suggested for the same purpose, the F_N and F_{NHi} circles are treated as circular bands, the width of each band being related to the error in the appropriate structure factor. A comprehensive set of modifications suggested by Green (1979) treats different types of errors separately. In particular, errors arising from imperfect isomorphism are treated in a comprehensive manner.

Although the isomorphous replacement method still remains the method of choice for the *ab initio* determination of protein structures, additional items of phase information from other sources are increasingly being used to replace, supplement, or extend the information obtained through the application of the isomorphous replacement. Methods have been developed for the routine refinement of protein structures (Watenpaugh *et al.*, 1973; Huber *et al.*, 1974; Sussman *et al.*, 1977; Jack & Levitt, 1978; Isaacs & Agarwal, 1978; Hendrickson & Konnert, 1980) and they provide a rich source of phase information. However, the nature of the problem and the inherent limitations of the Fourier technique are such that the possibility of refinement yielding misleading results exists (Vijayan, 1980*a*,*b*). It is therefore sometimes desirable to combine the phases obtained during refinement with the original isomorphous replacement phases. The other sources of phase information include molecular replacement (see Chapter 2.3), direct methods (Hendrickson & Karle, 1973; Sayre, 1974; de Rango *et al.*, 1975; see also Chapter 2.2) and different types of electron-density modifications (Hoppe & Gassmann, 1968; Collins, 1975; Schevitz *et al.*, 1981; Bhat & Blow, 1982; Agard & Stroud, 1982; Cannillo *et al.*, 1983; Raghavan & Tulinsky, 1979; Wang, 1985).

The problem of combining isomorphous replacement phases with those obtained by other methods was first addressed by Rossmann & Blow (1961). The problem was subsequently examined by Hendrickson & Lattman (1970) and their method, which involves a modification of the Blow and Crick formulation, is perhaps the most widely used for combining phase information from different sources.

The Blow and Crick procedure is based on an assumed Gaussian 'lumped' error in F_{NHi} which leads to a lack of closure, $\xi_{Hi}(\alpha)$, in F_{NHi} defined by (2.4.4.20). Hendrickson and Lattman make an equally legitimate assumption that the lumped error, again assumed to be Gaussian, is associated with F^2_{NHi}. Then, as in (2.4.4.20), we have

$$\xi''_{Hi}(\alpha) = F^2_{NHi} - D^2_{Hi}(\alpha), \qquad (2.4.4.33)$$

where $\xi''_{Hi}(\alpha)$ is the lack of closure associated with F^2_{NHi} for an assumed protein phase angle α. Then the probability for α being the correct phase angle can be expressed as

$$P_i(\alpha) = N_i \exp[-\xi''^2_{Hi}(\alpha)/2E''^2_i], \qquad (2.4.4.34)$$

where E''_i is the r.m.s. error in F^2_{NHi}, which can be evaluated using methods similar to those employed for evaluating E_i. Hendrickson and Lattman have shown that the exponent in the probability expression (2.4.4.34) can be readily expressed as a linear combination of five terms in the following manner.

$$-\xi''^2_{Hi}(\alpha)/2E''^2_i = K_i + A_i \cos\alpha + B_i \sin\alpha + C_i \cos 2\alpha + D_i \sin 2\alpha, \qquad (2.4.4.35)$$

where K_i, A_i, B_i, C_i and D_i are constants dependent on F_N, F_{Hi}, F_{NHi} and E''_i. Thus, five constants are enough to store the complete probability distribution of any reflection. Expressions for the five constants have been derived for phase information from anomalous scattering, tangent formula, partial structure and molecular replacement. The combination of the phase information from all sources can then be achieved by simply taking the total value of each constant. Thus, the total probability of the protein phase angle being α is given by

$$P(\alpha) = \prod P_s(\alpha) = N \exp\left(\sum_s K_s + \sum_s A_s \cos\alpha + \sum_s B_s \sin\alpha + \sum_s C_s \cos 2\alpha + \sum_s D_s \sin 2\alpha\right), \qquad (2.4.4.36)$$

where K_s, A_s *etc.* are the constants appropriate for the sth source and N is the normalization constant.

2.4.4.8. *Fourier representation of anomalous scatterers*

It is often useful to have a Fourier representation of only the anomalous scatterers in a protein. The imaginary component of the electron-density distribution obviously provides such a representation. When the structure is known and $F_N(+)$ and $F_N(-)$ have been experimentally determined, Chacko & Srinivasan (1970) have shown that this representation is obtained in a Fourier synthesis with $i[\mathbf{F}_N(+) + \mathbf{F}^*_N(-)]/2$ as coefficients, where $\mathbf{F}^*_N(-)$, whose magnitude is $F_N(-)$, is the complex conjugate of $\mathbf{F}_N(+)$. They also indicated a method for calculating the phase angles of $\mathbf{F}_N(+)$ and $\mathbf{F}^*_N(-)$. It has been shown (Hendrickson & Sheriff, 1987) that the Bijvoet-difference Fourier synthesis proposed earlier by Kraut (1968) is an approximation of the true imaginary component of the electron density. The imaginary synthesis can be useful in identifying minor anomalous-scattering centres when the major centres are known and also in providing an independent check on the locations of anomalous scatterers and in distinguishing between anomalous scatterers with nearly equal atomic numbers (Sheriff & Hendrickson, 1987; Kitagawa *et al.*, 1987).

2.4.5. Anomalous scattering of neutrons and synchrotron radiation. The multiwavelength method

The multiwavelength anomalous-scattering method (Ramaseshan, 1982) relies on the variation of dispersion-correction terms as a function of the wavelength used. The success of the method therefore depends upon the size of the correction terms and the availability of incident beams of comparable intensities at different appropriate wavelengths. Thus, although this method was used as early as 1957 (Ramaseshan *et al.*, 1957) as an aid to structure solution employing characteristic X-rays, it is, as outlined below, ideally suited in structural work employing neutrons and synchrotron radiation. In principle, γ-radiation can also be used for phase

references