resolution of the electron-microscope image, if one seeks phases for diffraction data in reciprocal-space regions where the objective lens phase contrast transfer function $|C(s)| \leq 0.2$, the method proves to be successful. The method is also quite effective for phase extension from 2 Å to 1 Å diffraction resolution, where the low-angle data serve as a large initial phase set for the tangent formula. However, no useful results were found from an *ab initio* phase determination carried out solely with the electron-diffraction structure-factor magnitudes. Similar results were obtained when *RANTAN* was used to phase experimental data from this compound (Fan *et al.*, 1991), *i.e.* the multisolution approach worked well for phase extension but not for *ab initio* phase determination. Additional tests were subsequently carried out with *QTAN* on an experimental *hk*0 electron-diffraction data set collected at 1200 kV (Dorset, McCourt, Fryer *et al.*, 1994). Again, *ab initio* phase determination is not possible by this technique. However, if a basis set was constructed from the Fourier transform of a 2.4 Å image, a correct solution could be found, but not at the lowest value of NQEST. This figure of merit was useful, however, when the basis set was taken from the symbolic addition determination mentioned in the previous section.

2.5.8.5. Density modification

Another method of phase determination, which is best suited to refining or extending a partial phase set, is the Hoppe–Gassmann density modification procedure (Hoppe & Gassmann, 1968; Gassmann & Zechmeister, 1972; Gassmann, 1976). The procedure is very simple but also very computer-intensive. Starting with a small set of (phased) $F_{\mathbf{h}}$, an initial potential map $\varphi(\mathbf{r})$ is calculated by Fourier transformation. This map is then modified by some real-space function, which restricts peak sizes to a maximum value and removes all negative density regions. The modified map $\varphi'(\mathbf{r})$ is then Fourier-transformed to produce a set of phased structure factors. Phase values are accepted *via* another modification function in reciprocal space, *e.g.* $E_{\rm calc}/E_{\rm obs} \geq p$, where p is a threshold quantity. The new set is then transformed to obtain a new $\varphi(\mathbf{r})$ and the phase refinement continues iteratively until the phase solution converges (judged by lower crystallographic R values).

The application of density modification procedures to electron-crystallographic problems was assessed by Ishizuka *et al.* (1982), who used simulated data from copper perchlorophthalocyanine within the resolution of the electron-microscope image. The method was useful for finding phase values in reciprocal-space regions where the transfer function $|C(s)| \leq 0.2$. As a technique for phase extension, density modification was acceptable for test cases where the resolution was extended from 1.67 to 1.0 Å, or 2.01 to 1.21 Å, but it was not very satisfactory for a resolution enhancement from 2.5 to 1.67 Å. There appear to have been no tests of this method yet with experimental data. However, the philosophy of this technique will be met again below in the description of the maximum entropy and likelihood procedure.

2.5.8.6. Convolution techniques

One of the first relationships ever derived for phase determination is the Sayre (1952) equation:

$$F_{\mathbf{h}} = \frac{\theta}{V} \sum_{\mathbf{k}} F_{\mathbf{k}} F_{\mathbf{h}-\mathbf{k}},$$

which is a simple convolution of phased structure factors multiplied by a function of the atomic scattering factors. For structures with nonoverlapping atoms, consisting of one atomic species, it is an exact expression. Although the convolution term resembles part of the tangent formula above, no statistical averaging is implied (Sayre, 1980). In X-ray crystallography this relationship has not been used very often, despite its accuracy. Part of the reason for this is that it requires relatively high resolution data for it to be useful. It can also fail for structures comprised of different atomic species.

Since, relative to X-ray scattering factors, electron scattering factors span a narrower range of magnitudes at $\sin\theta/\lambda = 0$, it might be thought that the Sayre equation would be particularly useful in electron crystallography. In fact, Liu *et al.* (1988) were able to extend phases for simulated data from copper perchlorophthalocyanine starting at the image resolution of 2 Å and reaching the 1 Å limit of an electron-diffraction data set. This analysis has been improved with a 2.4 Å basis set obtained from the Fourier transform of an electron micrograph of this material at 500 kV and extended to the 1.0 Å limit of a 1200 kV electron-diffraction pattern (Dorset *et al.*, 1995). Using the partial phase sets for zonal diffraction data from several polymers by symbolic addition (see above), the Sayre equation has been useful for extending into the whole *hk*0 set, often with great accuracy. The size of the basis set is critical but the connectivity to access all reflections is more so. Fan and co-workers have had considerable success with the analysis of incommensurately modulated structures. The average structure (basis set) is found by high-resolution electron microscopy and the 'superlattice' reflections, corresponding to the incommensurate modulation, are assigned phases in hyperspace by the Sayre convolution. Examples include a high T_c superconductor (Mo *et al.*, 1992) and the mineral ankangite (Xiang *et al.*, 1990). Phases of regular inorganic crystals have also been extended from the electron micrograph to the electron-diffraction resolution by this technique (Hu *et al.*, 1992).

In an investigation of how direct methods might be used for phase extension in protein electron crystallography, low-resolution phases from two proteins, bacteriorhodopsin (Henderson *et al.*, 1986) and halorhodopsin (Havelka *et al.*, 1993) were extended to higher resolution with the Sayre equation (Dorset *et al.*, 1995). For the noncentrosymmetric bacteriorhodopsin *hk*0 projection a 10 Å basis set was used, whereas a 15 Å set was accepted for the centrosymmetric halorhodopsin projection. In both cases, extensions to 6 Å resolution were reasonably successful. For bacteriorhodopsin, for which data were available to 3.5 Å, problems with the extension were encountered near 5 Å, corresponding to a minimum in a plot of average intensity *versus* resolution. Suggestions were made on how a multisolution procedure might be successful beyond this point.

2.5.8.7. Maximum entropy and likelihood

Maximum entropy has been applied to electron crystallography in several ways. In the sense that images are optimized, the entropy term

$$S = -\sum_i P_i \ln P_i,$$

where $P_i = p_i / \sum_i p_i$ and p_i is a pixel density, has been evaluated for various test electron-microscope images. For crystals, the true projected potential distribution function is thought to have the maximum value of S. If the phase contrast transfer function used to obtain a micrograph is unknown, test images (*i.e.* trial potential maps) can be calculated for different values of $\Delta f_{\rm trial}$. The value that corresponds to the maximum entropy would be near the true defocus. In this way, the actual objective lens transfer function can be found for a single image (Li, 1991) in addition to the other techniques suggested by this group.

Another use of the maximum-entropy concept is to guide the progress of a direct phase determination (Bricogne & Gilmore, 1990; Gilmore *et al.*, 1990). Suppose that there is a small set H of known phases $\phi_{\mathbf{h}\in H}$ (corresponding either to origin