This procedure was termed the 'indirect Fourier transformation (IFT)' method by Glatter (1979), who uses equidistant B splines in real space that are correlated by a Lagrange parameter, thus reducing the number of independent parameters to be fitted. Errors in determining a residual flat background only affect the innermost spline at $r = 0$; the intensity at $Q = 0$ and the radius of gyration are not influenced by a (small) flat background.

Another IFT method was introduced by Moore (1980), who uses an orthogonal set of sine functions in real space. This procedure is more sensitive to the correct choice of $D_{\max}$ and to a residual background that might be present in the data.

A major advantage of IFT is the ease with which the deconvolution of the scattering intensities with respect to the wavelength distribution and to geometrical smearing due to the primary beam and sample sizes is calculated by smearing the theoretical scattering curve obtained from the real-space model. In fact, it is possible to convolute the scattering curves obtained from the single splines that are calculated only once at the beginning of the fit procedure. The convoluted constituent curves are then iteratively fitted to the experimental scattering curves.

With the exception of particle symmetry, which is better seen in the scattering curve, structural features are more easily recognized in the $p(r)$ function (Glatter, 1982*a*).

Once the $p(r)$ function is determined, the zero-angle intensity and the radius of gyration can be calculated from its integral and from its second moment, respectively.

2.6.2.7.4. *Particle-size distribution*

Indirect Fourier transformation also facilitates the evaluation of particle-size distributions on the assumption that all particles have the same shape and that the size distribution depends on only one parameter (Glatter, 1980).

2.6.2.7.5. *Model fitting*

As in small-angle X-ray scattering, the scattering curves can be compared with those of simple or more elaborate models. This is rather straightforward in the case of highly symmetric particles like icosahedral viruses that can be regarded as spherical at low resolution. The scattering curves of such viruses are easily adapted by spherical-shell models assigning different scattering-length densities to the different shells (*e.g.* Cusack, 1984). Neutron constrast variation helps decisively to distinguish between the shells.

Fitting complicated models to the scattering curves is more critical because of the averaging effect of small-angle scattering. While it is correct and easy to show that the scattering curve produced by a model body coincides with the measured curve, in general a unique model cannot be deduced from the scattering curve alone. Stuhrmann (1970) has presented a procedure using Lagrange polynomials to calculate low-resolution real-space models directly from the scattering information. It has been applied successfully to the scattering curves from ribosomes (Stuhrmann *et al.*, 1976).

2.6.2.7.6. *Label triangulation*

Triangulation is one of the techniques that make full use of the advantages of neutron scattering. It consists in specifically labelling single components of a multicomponent complex, measuring the scattering curves from (*a*) particles with two labelled components, (*b*) and (*c*) particles with either of the two components labelled, and (*d*) a (reference) particle that is not labelled at all. The comparison of the scattering from $(b) + (c)$ with that from $(a) + (d)$ yields information on the scattering originating exclusively from vectors combining volume elements in one component with volume elements in the other component.

From this scattering difference curve, the distances between the centres of mass of the components are obtained. A table of such distances yields the spatial arrangement of the components. If there are n components in the complex, at least $4n - 10$ for $n > 3$ distance values are needed to build this model: Three distances define a basic triangle, three more yield a basic tetrahedron, the handedness of which is arbitrary and has to be determined by independent means. At least four more distances are required to fix a further component in space. More than four distances are needed if the resulting tetrahedron is too flat.

Label triangulation is based on a technique developed by Kratky & Worthmann (1947) for determining heavy-metal distances in organometallic compounds by X-ray scattering, and was proposed originally by Hoppe (1972); Engelman & Moore (1972) first saw the advantage of neutrons. The need to mix preparations (*a*) plus (*d*) and (*b*) plus (*c*) for obtaining the desired scattering difference curve in the case of high concentrations and/or inhomogeneous complexes (consisting of different classes of matter) has been shown by Hoppe (1973). The complete map of all protein positions within the small subunit from *E. coli* ribosomes has been obtained with this method (Capel *et al.*, 1987). An alternative approach for obtaining the distance information contained in the scattering curves from pairs of proteins by fitting the Fourier transform of 'moving splines' to the scattering curves has been presented by May & Nowotny (1989) for data on the large ribosomal subunit.

The scattering curves should be measured at the scattering-length-density matching point of the reference particle for reducing undesired contributions. Naturally inhomogeneous particles can be rendered homogeneous by specific partial deuteration. This technique has been successfully applied for ribosomes (Nierhaus *et al.*, 1983).

2.6.2.7.7. *Triple isotropic replacement*

An elegant way of determining the structure of a component inside a molecular complex has been proposed by Pavlov & Serdyuk (1987). It is based on measuring the scattering curves from three preparations. Two contain the complex to be studied at two different levels of labelling, ρ_1 and ρ_2, and are mixed together to yield sample 1, the third contains the complex at an intermediate level of labelling, ρ_3 (sample 2). If the condition

$$\rho_3(\mathbf{r}) = (1 - \delta)\rho_1(\mathbf{r}) + \delta\rho_2(\mathbf{r}) \qquad (2.6.2.13)$$

is satisfied by δ, the relative concentration of particle 2 in sample 1, then the difference between the scattering from the two samples only contains contributions from the single component. Additionally, the contributions from contamination, aggregation, and interparticle effects are suppressed provided that they are the same in the three samples, *i.e.* independent of the partial-deuteration states.

In the case of small complexes, δ can be obtained by measuring the scattering curves $I_1(Q)$, $I_2(Q)$, and $I_3(Q)$ of the three particles as a function of contrast and by plotting the differences of the zero-angle scattering $I_1(0) - I_3(0)$ and $I_2(0) - I_3(0)$ *versus* δ. The two curves intercept at the correct ratio δ_0.

The method, which can be considered as a special case of a systematic inverse contrast variation of a selected component, holds if the concentrations, the complex occupations, and the aggregation behaviour of the three particles are identical. Mathematically, the difference curve is independent of the

references