can be interpreted as a residual constant background (including the self-term of the constant nuclear 'form factor'), which may be used for slightly correcting the estimated background and consequently improving the quality of the data. For monodispersed particles, a particle surface can be deduced from the overall surface. The value of the surface area so determined depends on the maximal Q to which the scattering curve can be obtained with good statistics. This depends also on the magnitude of the background. At least for weakly scattering particles in mixtures of ^1H_2O and ^2H_2O, and even more in pure ^1H_2O, the incoherent background level often cannot be determined precisely enough for interpreting the tail of the scattering curve in terms of the surface area.

2.6.2.7. *Single-particle scattering*

Single-particle scattering in this context means scattering from isolated structures (clusters in alloys, isolated polymer chains in a solvent, biological macromolecules, *etc*.) randomly distributed in space and sufficiently far away from each other so that interparticle contributions to the scattering (see Subsection 2.6.2.8) can be neglected. The tendency of polymerization of single particles, for example the monomer–dimer equilibrium of proteins or the formation of higher aggregates, and long-range (*e.g.* electrostatic) interactions between the particles disturb single-particle scattering. In the absence of such effects, samples with solute volume fractions below about 1% can be regarded as free of volume-exclusion interparticle effects for most purposes. For (monodispersed) protein samples, for example, this means that concentrations of about $5\,\mathrm{mg\,ml^{-1}}$ are often a good compromise between sufficient scattering intensity and concentration effects. In many cases, series of scattering measurements with increasing particle concentrations have been used for extrapolating the scattering to zero concentration. In the following, we assume that particle interactions are absent.

2.6.2.7.1. *Particle shape*

All X-ray and neutron small-angle scattering curves can be approximated by a parabolic fit in a narrow Q range near $Q = 0$ (Porod, 1951): $I(Q) \simeq I(0)\ (1 - a^2Q^2/3 + \ldots)$. In the case of single-particle scattering, a Gaussian approximation to the scattering curve is even more precise (Guinier & Fournet, 1955) in the zero-angle limit:

$$I(Q) \simeq I(0)\exp(-Q^2/3R_G^2), \qquad (2.6.2.10)$$

where R_G is the radius of gyration of the particle's excess scattering density.

The concept of R_G and the validity of the Guinier approximation is discussed in more detail in the SAXS section of this volume (§2.6.1). It might be mentioned here that the frequently used $QR_G < 1$ rule for the validity of the Guinier approximation is no more than an indication and should always be tested by a scattering calculation with the model obtained from the experiment: Spheres yield a deviation of 5% of the Gaussian approximation at $QR_G = 1.3$, rods at $QR_G = 0.6$; ellipsoids of revolution with an elongation factor of 2 can reach as far as $QR_G = 3$.

More detailed shape information requires a wider Q range. As indicated before, Fourier transforms may help to distinguish between conflicting models. In many instances (*e.g.* hollow bodies, cylinders), it is much easier to find the shape of the scattering particle from the distance distribution function than from the scattering curve [see §2.6.2.7.3].

2.6.2.7.2. *Particle mass*

With $N = CN_AV_s/M_r$, where N_A is Avogadro's number, C is the mass concentration of the solute in $\mathrm{g\,l^{-1}}$, and V_s is the sample volume in $\mathrm{cm^{-3}}$ (we assume N identical particles randomly distributed in dilute solution), we find that the relative molecular mass M_r of a particle can be determined from the intensity at zero angle, $I(0)$ in equation (2.6.2.10), using the relation (Jacrot & Zaccai, 1981), where the particle mass concentration C (in $\mathrm{mg\,ml^{-1}}$) is omitted:

$$I(0)/\{CI[\mathrm{H_2O}](0)\} = 4\pi f\, T_sM_rN_Ad_s\, 10^{-3}\left[\left(\sum b_i - \rho_sV\right)/M_r\right]^2/(1 - T[\mathrm{H_2O}]). \qquad (2.6.2.11)$$

d_s is the sample thickness. Note that $\sum b_i/M_r$ may depend on solvent exchange; in a given solvent, especially ^1H_2O, it is rather independent of the exact amino acid composition of proteins (Jacrot & Zaccai, 1981).

An alternative presentation of equation (2.6.2.11) is

$$I(0)/\{CI[\mathrm{H_2O}](0)\} = 4\pi f\, T_sM_rd_s\ 10^{-3}(v\Delta\rho)^2/N_A(1 - T[\mathrm{H_2O}]), \qquad (2.6.2.11a)$$

where $\Delta\rho = \rho_p(\rho_s) - \rho_s$ is the contrast; ρ_p is the particle scattering-length density (depending on the scattering-length density ρ_s of the solvent, in general) and v is the partial specific volume of the particle. Expression (2.6.2.11*a*) is of advantage when $(v\Delta\rho)$, which is a linear function of ρ_s, is known for a class of particles.

A thermodynamic approach to the particle-size problem, in view of the complementarity of different methods, has been given Zaccai, Wachtel & Eisenberg (1986) on the basis of the theory of Eisenberg (1981). It permits the determination of the molecular mass, of the hydration, and of the amount of bound salts.

2.6.2.7.3. *Real-space considerations*

The scattering from a large number of randomly oriented particles at infinite dilution, and as a first approximation that of particles at sufficiently high dilution (see above), is completely determined by a function $p(r)$ in real space, the distance-distribution function. It describes the probability p of finding a given distance r between any two volume elements within the particle, weighted with the product of the scattering-length densities of the two volume elements.

Theoretically, $p(r)$ can be obtained by an infinite sine Fourier transform of the isolated-particle scattering curve

$$I(Q) = \int_0^\infty [p(r)/Qr]\sin(Qr)\,\mathrm{d}r. \qquad (2.6.2.12)$$

In practice, the scattering curve can be measured neither to $Q = 0$ (but an extrapolation is possible to this limit), nor to $Q \to \infty$. In fact, neutrons allow us to measure more easily the sample scattering in the range near $Q = 0$; X-rays are superior for large Q values. Indirect iterative methods have been developed that fit the finite Fourier transform

$$I(Q) = \int_0^{D_{\max}} [p(r)/Qr]\sin(Qr)\,\mathrm{d}r \qquad (2.6.2.12a)$$

of a $p(r)$ function described by a limited number of parameters between $r = 0$ and a maximal chord length $D_{\max}$ within the particle to the experimental scattering curve. It differs from the $p(r)$ of Section 2.6.1 by a factor of 4π.

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

contrast of the rest of the complex with respect to the solvent. In practice, it would be wise to follow the same considerations as with triangulation.

2.6.2.8. *Dense systems*

Especially in the case of polymers, but also in alloys, the scattering from the sample can often no longer be described, as in the previous section, as originating from a sum of isolated particles in different orientations. There may be two reasons for this: either the number concentration c of one of the components is higher than about 0.01, leading to excluded-volume effects, and/or there is an electrostatic interaction between components (for example, in solutions of polyelectrolytes, latex, or micelles). In these cases, it is usually the information about the *structure* of the sample caused by the interactions that is to be obtained rather than the shape of the inhomogeneities or particles in the sample, unless the interactions can be regarded as a weak disturbance.

An excellent introduction to the treatment of dense systems is found in the article of Hayter (1985). A detailed description of the theoretical interpretation of correlations in charged macromolecular and supramolecular solutions has been published by Chen, Sheu, Kalus & Hoffmann (1988).

The scattering from densely packed particles can be written as the product of the structure factor or structure function $S(Q)$, describing the arrangement of the inhomogeneities with respect to each other, in mathematical terms the interference effects of correlations between particle positions, in the sample,

$$S(Q) = \left\langle \sum \sum \exp[i\mathbf{Q}(\mathbf{r}_j - \mathbf{r}_k)] \right\rangle / N, \tag{2.6.2.14}$$

and of the form factor $P(Q)$ of the inhomogeneities (as before):

$$I(Q) = P(Q)S(Q). \tag{2.6.2.15}$$

Hayter & Penfold (1981) were the first to describe an analytic structure factor for macro-ion solutions.

If $P(Q)$ can be obtained from a measurement of a dilute solution of the particles under study, then the pure structure factor can be calculated by dividing the high-concentration intensity curve by the low-concentration curve. This procedure requires the form factor not to change with concentration, which is not necessarily the case for loosely arranged particles such as polymers. A technique that avoids this problem is contrast variation (see Subsection 2.6.2.2): introducing a fraction of a deuterated molecule into a bulk of identical protonated molecules (or *vice versa*, with the advantage of reduced incoherent background) yields the scattering of the 'isolated' labelled particle at high-concentration conditions.

Partial structure factors can be obtained from a contrast-variation series of a given system at different volume fractions of the particles. Similarly to equation (2.6.2.4), the structure factor can be decomposed into a quadratic function. In the ternary alloy Al–Ag–Zn, for example, the scattering has been decomposed into the contributions from the two minor species Ag and Zn, and their interference, *i.e.* the partial structure functions for Zn–Zn, Zn–Ag, and Ag–Ag, by using the scattering from three samples with different silver isotopes, and identical sample treatment (Salva-Ghilarducci, Simon, Guyot & Ansara, 1983).

references