2.6. SMALL-ANGLE TECHNIQUES

surface scattering by scratches. The vanadium sample has to be hermetically sealed to prevent hydrogen incorporation (Wignall & Bates, 1987).

The coherent cross sections of the two protons and one oxygen in light water add up to a nearly vanishing *coherent*-scattering-length density, whereas the incoherent scattering length of the water molecule remains very high. The (quasi)isotropic incoherent scattering from a thin, *i.e.* about 1 mm or less, sample of ^1H_2O, therefore, is an ideal means for determining the absolute intensity of the sample scattering (Jacrot, 1976; Stuhrmann *et al.*, 1976), on condition that the sample-to-detector distance L is not too large, *i.e.* up to about 10 m. A function $f(\sigma_t[H_2O], \lambda)$ that accounts for deviations from the isotropic behaviour due to inelastic incoherent-scattering contributions of ^1H_2O and for the influence of the wavelength dependence of the detector response has to be multiplied to the right-hand side of equation (2.6.2.6) (May, Ibel & Haas, 1982). f can be determined experimentally and takes values of around 1 for wavelengths around 1 nm.

Since the intensity scattered into a solid angle $\Delta\Omega$ is

$$I(Q) = P(Q)NT_s I_0 g \left(\sum b_i - \rho_s V \right)^2, \qquad (2.6.2.7)$$

where $P(Q)$ is the form factor of the scattering of one particle, and the geometrical factor g can be chosen so that it is the same as that of equation (2.6.2.6) (same sample thickness and surface and identical collimation conditions), we obtain

$$I(Q) = 4\pi P(Q)NT_s f(\sigma_t[H_2O], \lambda)\left(\sum b_i - \rho_s V\right)^2/(1 - T[H_2O]). \qquad (2.6.2.8)$$

Note that the scattering intensities mentioned above are scattering intensities corrected for container scattering, electronic and neutron background noise, and, in the case of the sample, for the solvent scattering.

2.6.2.6.2. *Detector-response correction*

For geometrical reasons (*e.g.* sample absorption), and in the case of 2D detectors also for electronic reasons, the scattering curves cannot be measured with a sensitivity uniform over all the angular region. Therefore, the scattering curve has to be corrected by that of a sample with identical geometrical properties, but scattering the neutrons with the same probability into all angles (at least in the forward direction). As we have seen previously, such samples are vanadium and thin cells filled with light water. Again, water has the advantage of a much higher scattering cross section, and is less influenced by surface effects.

At large sample-to-detector distances (more than about 10 m), the scattering from water is not sufficiently strong to enable its use for correcting sample scattering curves obtained with the same settings. Experience shows that it is possible in this case to use a water scattering curve measured at a shorter sample-to-detector distance. This should be sufficiently large not to be influenced by the deviation of the (flat) detector surface from the spherical shape of the scattered waves and small enough so that the scattering intensity per detector element is still sufficient, for example about 3 m. It is necessary to know the intensity loss factor due to the different solid angles covered by the detector element and by the apparent source in both cases. This can be determined, for instance, by comparing the global scattering intensity of water on the whole detector for both conditions (after correction for the background scattering) or from the intensity shift of the same sample measured at both detector distances in a plot of the logarithm of the intensity *versus* Q.

2.6.2.6.3. *Estimation of the incoherent scattering level*

For an exact knowledge of the scattering curve, it is necessary to subtract the level of incoherent scattering from the scattering curve, which is initially a superposition of the (desired) coherent sample scattering, electronic and neutron background noise, and (sometimes dominant) incoherent scattering.

A frequently used technique is the subtraction of a reference sample that has the same level of incoherent scattering, but lacks the coherent scattering from the inhomogeneities under study. Although this seems simple in the case of solutions, in practice there are problems: Very often, the $^1H/^2H$ mixture is made by dialysis, and the last dialysis solution is taken as the reference. The dialysis has to be excessive to obtain really identical levels of ^1H, and in reality there is often a disagreement that is more important the lower the sample concentration is. If the concentration is high, then the incoherent scattering from the sample atoms (protons) themselves becomes important.

For dilute aqueous solutions, there is a procedure using the sample and reference transmissions for estimating the incoherent background level (May, Ibel & Haas, 1982): The incoherent scattering level from the sample, $I_{i,s}$, can be estimated as

$$I_{i,s} = I[H_2O]f_\lambda(1 - T_s)/(1 - T[H_2O]), \qquad (2.6.2.9)$$

where $I[H_2O]$ is the scattering from a water sample, $T[H_2O]$ is transmission, T_s that of the sample. f_λ is a factor depending on the wavelength, the detector sensibility, the solvent composition, and the sample thickness; it can be determined experimentally by plotting $I_{i,s}/I[H_2O]$ *versus* $(1 - T_s)/(1 - T[H_2O])$ for a number of partially deuterated solvent mixtures.

This procedure is justified because of the overwhelming contribution of the incoherent scattering of ^1H to the macroscopic scattering cross section of the solution, and therefore to its transmission. The procedure should also be valid for organic solvents. The precision of the estimation is limited by the precision of the transmission measurement, the relative error of which can hardly be much better than about 0.005 for reasonable measuring times and currently available equipment, and by the (usually small) contribution of the coherent cross section to the total cross section of the solution. A modified version of (2.6.2.9) can be used if a solvent with a transmission close to that of a sample has been measured, but the factor f_λ should not be omitted.

An equation similar to (2.6.2.9) holds for systems with a larger volume occupation c of particles in a (protonated) solvent with a scattering level I_{inc} in a cell with identical pathway (without the particles):

$$I_{i,s} = I_{inc}(1 - T_{inc}^{1-c})/(1 - T_{inc}). \qquad (2.6.2.9a)$$

In this approximation, the particles' cross-section contribution is assumed to be zero, *i.e.* the particles are considered as bubbles.

In the case of dilute systems of monodisperse particles, the residual background (after initial corrections) can be quite well estimated from the zero-distance value of the distance-distribution function calculated by the indirect Fourier transformation of Glatter (1979).

2.6.2.6.4. *Inner surface area*

According to Porod (1951, 1982), small-angle scattering curves behave asymptotically like $I(Q) = \text{constant} \times A_s Q^{-4}$ for large Q, where A_s is the inner surface of the sample. Theoretically, fitting a straight line to $I(Q)Q^4$ *versus* Q^4 ('Porod plot') *at sufficiently large* Q therefore yields a zero intercept, which is proportional to the internal surface; a slope

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 mono-dispersed 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 $5\,mg\,ml^{-1}$ 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): $I(Q) \simeq I(0)\,(1 - a^2 Q^2/3 + \ldots)$. 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 $N = CN_A V_s/M_r$, where N_A is Avogadro's number, C is the mass concentration of the solute in $g\,l^{-1}$, and V_s is the sample volume in 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 $mg\,ml^{-1}$) is omitted:

$$I(0)/\{CI[H_2O](0)\}$$
$$= 4\pi f\,T_s M_r N_A d_s\,10^{-3}\big[\big(\textstyle\sum b_i - \rho_s V\big)/M_r\big]^2/(1 - T[H_2O]).$$
$$(2.6.2.11)$$

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

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

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 (2.6.2.11a) 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

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

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

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

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.