2.6. SMALL-ANGLE TECHNIQUES

2.6.2. Neutron techniques (By R. May)

Symbols used in the text

A	sample area
A_s	inner sample surface
b_i	coherent scattering length of atom i
B_i	spin-dependent scattering length of atom i
C	sample concentration in $\mathrm{g\,l^{-1}}$
c	volume fraction occupied by matter
d	sample thickness
D	particle dimension
DCD	double-crystal diffractometer
d_0	Bragg spacing
$\mathbf{e}, \mathbf{e}_0$	unit vectors along the diffracted and incident beams
$\mathbf{I}$	nuclear spin
IFT	indirect Fourier transformation
N	number of particles in the sample
N_A	Avogadro's number
Q	momentum transfer $[= (4\pi/\lambda)\sin\theta]$
r	radius of a sphere
R_G	radius of gyration
$\mathbf{s}$	neutron spin
SANS	small-angle neutron scattering
SAXS	small-angle X-ray scattering
T	transmission
TOF	time of flight
v	partial specific volume
V_p	particle volume
V_s	sample volume
$\Delta\Omega$	solid angle subtended by a detection element
λ	wavelength
ρ	scattering-length density
2θ	full scattering angle
$\mathrm{d}\sigma(Q)/\mathrm{d}\Omega$	scattering cross section per particle and unit solid angle

2.6.2.1. *Relation of X-ray and neutron small-angle scattering*

X-ray and neutron small-angle scattering (SAXS and SANS, respectively) are dealing with the same family of problems, *i.e.* the investigation of 'inhomogeneities' in matter. These inhomogeneities have dimensions D of the order of 1 to 100 nm, which are larger than interatomic distances, *i.e.* 0.3 nm. The term inhomogeneities may mean clusters in metals, a small concentration of protonated chains in an otherwise identical deuterated polymer – or *vice versa* – but also particles as well defined as purified proteins in aqueous solution.

In most cases, the inhomogeneities are not ordered. This is where small-angle scattering is most useful: many systems are not crystalline, cannot be crystallized, or do not exhibit the same properties if they are. One field, if one may say so, of SANS where samples are ordered is low-resolution crystallography of biological macromolecules. It will not be treated further here. In the case of crystalline order, the scattering of the single particle is observed with an amplification factor of N^2 for N identical particles in the crystal, but only for those scattering vectors observing the Bragg condition $n\lambda = 2d_0\sin\theta$. For disordered, randomly oriented particles, the amplification is only N, and the scattering pattern is lacking all information on particle orientation. Moreover, the real-space information on the internal arrangement of atoms within the inhomogeneities is reduced to the 'distance distribution function', a sine Fourier transform of the scattering intensity.

The mathematical descriptions of SAXS and SANS are either identical or hold with equivalent terms. The reader is referred to Section 2.6.1 on X-ray small-angle scattering techniques for a general description of low-Q scattering. An abundant treatment of SAXS can be found in the book edited by Glatter & Kratky (1982), and in Guinier & Fournet (1955) and Guinier (1968). A general introduction to SANS is given, for example, by Kostorz (1979) and by Hayter (1985). This section deals mainly with the differences between the techniques.

Altogether, neutrons are used for low-Q scattering essentially for the same reasons as for other neutron experiments. These reasons are:

(1) neutrons are sensitive to the isotopic composition of the sample;

(2) neutrons possess a magnetic moment and, therefore, can be used as a magnetic probe of the sample; and

(3) because of their weak interaction with and consequent deep penetration into matter, neutrons allow us to investigate properties of the bulk;

(4) for similar reasons, strong transparent materials are available as sample-environment equipment.

The fact that the kinetic energies of thermal and cold neutrons are comparable to those of excitations in solids, which is a reason for the use of neutrons for inelastic scattering, is, with the exception of time-of-flight SANS (see §2.6.2.1.1), not of importance for SANS.

The information obtained from low-Q scattering is always an average over the irradiated sample volume and over time. This average may be purely static (in the case of solids) or also dynamic (liquids). The limited Q range used does not resolve interatomic scattering contributions. Thus, a 'scattering-length density' ρ can be introduced, $\rho = \sum b_i/V$, where b_i are the (coherent) scattering lengths of the atoms within a volume V with linear dimensions of at least λ/π. Inhomogeneities can then be understood as regions where the scattering-length density deviates from the prevailing average value.

2.6.2.1.1. *Wavelength*

In the case of SANS – as in that of X-rays from synchrotron sources – the wavelength dependence of the momentum transfer Q, $Q = (4\pi/\lambda)\sin\theta$, where θ is half the scattering angle and λ is the wavelength, has to be taken into account explicitly. Q corresponds to k, h, and $2\pi s$ used by other authors.

SANS offers an optimal choice of the wavelength: with sufficiently large wavelengths, for example, first-order Bragg scattering (and therefore the contribution of multiple Bragg scattering to small-angle scattering) can be suppressed: The Bragg condition written as $\lambda/d_{\max} = (2\sin\theta)/n < 2$ cannot hold for $\lambda \geq 2d_{\max}$, where $d_{\max}$ is the largest atomic distance in a crystalline sample. For the usually small scattering angles in SANS, even quite small λ will not produce first-order peaks.

The neutrons produced by the fuel element of a reactor or by a pulsed source are moderated by the (heavy) water surrounding the core. Normally, the neutrons leave the reactor with a thermal velocity distribution. Cold sources, small vessels filled with liquid deuterium in the reactor tank, permit the neutron velocity distribution to be slowed down ('cold' neutrons) and lead to neutron wavelengths (range 0.4 to 2 nm) which are more useful for SANS.

At reactors, a narrow wavelength band is usually selected for SANS either by an artificial-multilayer monochromator or – more frequently, owing to the slow speed of cold neutrons – by a velocity selector. This is a rotating drum with a large number (about 100) of helical slots at its circumference, situated at the entrance of the neutron guides used for collimation. Only neutrons of the suitable velocity are able to pass through this

drum. The wavelength resolution $\Delta\lambda/\lambda$ of velocity selectors is usually between 5 and 40% (full width at half-maximum, FWHM); 10 and 20% are frequently used values.

Alternatively, time-of-flight (TOF) SANS cameras have been developed on pulsed neutron sources (*e.g.* Hjelm, 1988). These use short bunches (about 100 μs long) of neutrons with a 'white' wavelength spectrum produced by a pulsed high-energy proton beam impinging on a target with a repetition rate of the order of 10 ms. The wavelength and, consequently, the Q value of a scattered neutron is determined by its flight time, if the scattering is assumed to be quasi-elastic. The dynamic Q range of TOF SANS instruments is rather large, especially in the high-Q limit, owing to the large number of rapid neutrons in the pulse. The low-Q limit is determined by the pulse-repetition rate of the source because of frame overlap with the following pulse. It can be decreased, if necessary with choppers turning in phase with the pulse production and selecting only every nth pulse. This disadvantage does not exist for reactor-based TOF SANS cameras, where the pulse-repetition rate can be optimally adapted to the chosen maximal and minimal wavelength. A principal problem for TOF SANS exists in the 'upscattering' of cold neutrons, *i.e.* their gain in energy, by ^{1}H-rich samples: The background scattering may not arrive simultaneously with the elastic signal, and may thus not be attributed to the correct Q value (Hjelm, 1988).

2.6.2.1.2. *Geometry*

With typical neutron wavelengths, low Q need not necessarily mean small angles: The interesting Q range for an inhomogeneity of dimension D can be estimated as $1/D < Q < 10/D$. The scattering angle corresponding to the upper Q limit for $D = 10$ nm is 1.4° for Cu $K\alpha$ radiation, but amounts to 9.1° for neutrons of 10 nm wavelength. Consequently, it is preferable to speak of low-Q rather than of small-angle neutron scattering.

'Pin-hole'-type cameras are the most frequently used SANS instruments; an example is the SANS camera D11 at the Institut Max von Laue–Paul Langevin in Grenoble, France (Ibel, 1976; Lindner, May & Timmins, 1992), from which some of the numbers below are quoted. Since the cross section of the primary beam is usually chosen to be rather large (*e.g.* 3×5 cm) for intensity reasons, pin-hole instruments tend to be large. The smallest Q value that can be measured at a given distance is just outside the image of the direct beam on the detector (which either has to be attenuated or is hidden behind a beamstop, a neutron-absorbing plate of several 10 cm^2, *e.g.* of cadmium). Very small Q values thus require long sample-to-detector distances. The area detector of D11, with a surface of 64×64 cm and resolution elements of 1 cm^2, moves within an evacuated tube of 1.6 m diameter and a length of 40 m. Thus, a Q range of 5×10^{-3} to 5 nm^{-1} is covered. The geometrical resolution is determined by the length of the free neutron flight path in front of the sample, moving sections of neutron guide into or out of the beam ('collimation'). In general, the collimation length is chosen roughly equal to the sample-to-detector distance. Thus, the geometrical and wavelength contributions to the Q resolution match at a certain distance of the scattered beam from the direct-beam position in the detector plane. In order to resolve scattering patterns with very detailed features, *e.g.* of particles with high symmetry, longer collimation lengths are sometimes required at the expense of intensity.

Much more compact double-crystal neutron diffractometers [described for X-rays by Bonse & Hart (1966)] are being used to reach the very small Q values of some 10^{-4} nm^{-1} typical of static light scattering. The sample is placed between two crystals. The first crystal defines the wavelength and the direction of the incoming beam. The other crystal scans the scattered intensity. The resolution of such an instrument is mainly determined by the Darwin widths of the ideal crystals. This fact is reflected in the low neutron yield. Slit geometry can be used, but not 2D detectors.

A recent development is the ellipsoidal-mirror SANS camera. The mirror, which needs to be of very high surface quality, focuses the divergent beam from a small (several mm^2) source through the sample onto a detector with a resolution of the order of 1×1 mm. Owing to the more compact beam image, all other dimensions of the SANS camera can be reduced drastically (Alefeld, Schwahn & Springer, 1989). Whether or not there is a gain in intensity as compared with pin-hole geometry is strongly determined by the maximal sample dimensions. Long mirror with cameras (*e.g.* 20 m) are always superior to double-crystal instruments in this respect (Alefeld, Schwahn & Springer, 1989), and can also reach the light-scattering Q domain (Q_{min} of some 10^{-4} nm^{-1}, corresponding to particles of several μm dimension).

2.6.2.1.3. *Correction of wavelength, slit, and detector-element effects*

Resolution errors affect SANS data in the same way as X-ray scattering data, for which one may find a detailed treatment in an article by Glatter (1982*b*); there is one exception to this; namely, gravity, which of course only concerns neutron scattering, and only in rare cases (Boothroyd, 1989). Since SANS cameras usually work with pin-hole geometry, the influences of the slit sizes, *i.e.* the effective source dimensions, on the scattering pattern are small; even less important is, in general, the pixel size of 2D detectors. The preponderant contribution to the resolution of the neutron-scattering pattern is the wavelength-distribution function after the monochromatizing device, especially at larger angles. The situation is more complicated for TOF SANS (Hjelm, 1988).

As has been shown in an analytical treatment of the resolution function by Pedersen, Posselt & Mortensen (1990), who also quote some relevant references, resolution effects have a small influence on the results of the data analysis for scattering patterns with a smooth intensity variation and without sharp features. Therefore, one may assume that a majority of SANS patterns are not subjected to desmearing procedures.

Resolution has to be considered for scattering patterns with distinct features, as from spherical latex particles (Wignall, Christen & Ramakrishnan, 1988) or from viruses (Cusack, 1984). Size-distribution and wavelength-smearing effects are similar; it is evident that wavelength effects have to be corrected for if the size distribution is to be obtained.

Since measured scattering curves contain errors and have to be smoothed before they can be desmeared, iterative indirect methods are, in general, superior: A guessed solution of the scattering curve is convoluted with known smearing parameters and iteratively fitted to the data by a least-squares procedure. The guessed solution can be a simply parameterized scattering curve, without knowledge of the sample (Schelten & Hossfeld, 1971), but it is of more interest to fit the smeared Fourier transform of the distance-distribution function (Glatter, 1979) or the radial density distribution (*e.g.* Cusack, Mellema, Krijgsman & Miller, 1981) of a real-space model to the data.

2.6.2.2. *Isotopic composition of the sample*

Unlike X-rays, which 'see' the electron clouds of atoms within a sample, neutrons interact with the point-like nuclei. Since their

form factor does not decay like the atomic form factor, an isotropic background from the nuclei is present in all SANS measurements.

While X-ray scattering amplitudes increase regularly with the atomic number, neutron coherent-scattering amplitudes that give rise to the interference scattering necessary for structural investigations vary irregularly (see Bacon, 1975). Isotopes of the same element often have considerably different amplitudes owing to their different resonant scattering. The most prominent example of this is the difference of the two stable isotopes of hydrogen, ^1H and ^2H (deuterium). The coherent-scattering length of ^2H is positive and of similar value to that of most other elements in organic matter, whereas that of ^1H is negative, *i.e.* for ^1H there is a $180°$ phase shift of the scattered neutrons with respect to other nuclei.

This latter difference has been exploited vastly in the fields of polymer science (*e.g.* Wignall, 1987) and structural molecular biology (*e.g.* Timmins & Zaccai, 1988), in mainly two complementary respects, contrast variation and specific isotopic labelling.

In the metallurgy field, other isotopes are being used frequently for similar purposes, for example the nickel isotope ^{62}Ni, which has a negative scattering length, and the silver isotopes ^{107}Ag and ^{109}Ag (see the review of Kostorz, 1988).

2.6.2.2.1. *Contrast variation*

The easiest way of using the scattering-amplitude difference between ^1H and ^2H is the so-called contrast variation. It was introduced into SANS by Ibel & Stuhrmann (1975) on the basis of X-ray crystallographic (Bragg & Perutz, 1952), SAXS (Stuhrmann & Kirste, 1965), and light-scattering (Benoit & Wippler, 1960) work. Most frequently, contrast variation is carried out with mixtures of light (^1H_2O) and heavy water (^2H_2O), but also with other solvents available in protonated and deuterated form (ethanol, cyclohexane, *etc.*). The scattering-length density of H_2O varies between -0.562×10^{10} cm^{-2} for normal water, which is nearly pure ^1H_2O, and 6.404×10^{10} cm^{-2} for pure heavy water.

The scattering-length densities of other molecules, in general, are different from each other and from pure protonated and deuterated solvents and can be matched by $^1H/^2H$ mixture ratios characteristic for their chemical compositions. This mixture ratio (or the corresponding absolute scattering-length density) is called the scattering-length-density match point, or, semantically incorrect, contrast match point. If a molecule contains non-covalently bound hydrogens, they can be exchanged for solvent hydrogens. This exchange is proportional to the ratio of all labile ^1H and ^2H present; in dilute aqueous solutions, it is dominated by the solvent hydrogens. A plot of the scattering-length density *versus* the $^2H/(^2H+^1H)$ ratio in the solvent shows a linear increase if there is exchange; the value of the match point also depends on solvent exchange. The fact that many particles have high contrast with respect to ^2H_2O makes neutrons superior to X-rays for studying small particles at low concentrations.

The scattered neutron intensity from N identical particles without long-range interactions in a (very) dilute solution with solvent scattering density ρ_s can be written as

$$I(Q) = [\,d\sigma(Q)/\,d\Omega]NTAI_0\Delta\Omega, \qquad (2.6.2.1)$$

with the scattering cross section per particle and unit solid angle

$$d\sigma(Q)/\,d\Omega = \left\langle \left| \int[\rho(\mathbf{r}) - \rho_s]\exp(i\mathbf{Q}\cdot\mathbf{r})\,d\mathbf{r}\right|^2 \right\rangle. \qquad (2.6.2.1a)$$

The angle brackets indicate averaging over all particle orientations. With $\rho(r) = \sum b_i/V_p$ and $I(0) = \text{constant} \times \left\langle \left| \int[\rho(\mathbf{r}) - \rho_s]\,d\mathbf{r}\right|^2 \right\rangle$, we find that the scattering intensity at zero angle is proportional to

$$\Delta\rho = \sum b_i/V_p - \rho_s, \qquad (2.6.2.2)$$

which is called the contrast. The exact meaning of V_p is discussed, for example, by Zaccai & Jacrot (1983), and for X-rays by Luzzati, Tardieu, Mateu & Stuhrmann (1976).

The scattering-length density $\rho(\mathbf{r})$ can be written as a sum

$$\rho(\mathbf{r}) = \rho_0 + \rho_F(\mathbf{r}), \qquad (2.6.2.3)$$

where ρ_0 is the average scattering-length density of the particle at zero contrast, $\Delta\rho = 0$, and $\rho_F(\mathbf{r})$ describes the fluctuations about this mean. $I(Q)$ can then be written

$$I(Q) = (\rho_0 - \rho_2)^2 I_c(Q) + (\rho_0 - \rho_s)I_{cs}(Q) + I_s(Q). \qquad (2.6.2.4)$$

I_s is the scattering intensity due to the fluctuations at zero contrast. The cross term $I_{cs}(Q)$ also has to take account of solvent-exchange phenomena in the widest sense (including solvent water molecules bound to the particle surface, which can have a density different from that of bulk water). This extension is mathematically correct, since one can assume that solvent exchange is proportional to $\Delta\rho$. The term I_c is due to the invariant volume inside which the scattering density is independent of the solvent (Luzzati, Tardieu, Mateu & Stuhrmann, 1976). This is usually not the scattering of a homogeneous particle at infinte contrast, if the exchange is not uniform over the whole particle volume, as is often the case, or if the particle can be imaged as a sponge (see Witz, 1983).

The method is still very valuable, since it allows calculation of the scattering at any given contrast on the basis of at least three measurements at well chosen $^1H/^2H$ ratios (including data near, but preferentially not exactly at, the lowest contrasts). It is sometimes limited by ^2H-dependent aggregation effects.

2.6.2.2.2. *Specific isotopic labelling*

Specific isotope labelling is a method that has created unique applications of SANS, especially in the polymer field. Again, it is mainly concerned with the exchange of ^1H by ^2H, this time in the particles to be studied themselves, at hydrogen positions that are not affected by exchange with solvent atoms, for example carbon-bound hydrogen sites.

With this technique, isolated polymer chains can be studied in the environment of other polymer chains which are identical except for the hydrogen atoms, which are either ^1H or ^2H. Even if some care has to be taken as far as slightly modified thermodynamics are concerned, there is no other method that could replace neutrons in this field.

Inverse contrast variation forms an intermediate between the two methods described above. The contrast with respect to the solvent of a whole particle or of well defined components of a particle, for example a macromolecular complex, is changed by varying its degree of deuteration. That of the solvent remains constant. Since solvent-exchange effects remain practically identical for all samples, the measurements can be more precise than in the classical contrast variation (Knoll, Schmidt & Ibel, 1985).

2.6.2.3. *Magnetic properties of the neutron*

Since the neutron possesses a magnetic moment, it is sensitive to the orientation of spins in the sample [see, for example, Abragam *et al.* (1982)]. Especially in the absence of any other (isotopic) contrast, an inhomogeneous distribution of spins in the

sample is detectable by neutron low-Q scattering. The neutron spins need not be oriented themselves, although important contributions can be expected from measuring the difference between the scattering of neutron beams with opposite spin orientation. At present, several low-Q instruments are being planned or even built including neutron polarization and polarization analysis.

Studies of magnetic SANS without (and rarely with) neutron polarization include dislocations in magnetic crystals and amorphous ferromagnets [see the review of Kostorz (1988)].

Janot & George (1985) have pointed out that it is important to apply contrast variation for suppressing surface-roughness scattering and/or volume scattering in order to isolate magnetic scattering contributions by matching the scattering-length density of the material with that of a mixture of heavy and light water or oil, *etc*.

2.6.2.3.1. *Spin-contrast variation*

For a long time, the magnetic properties of the neutron have been neglected as far as 'nonmagnetic' matter is concerned. Spin-contrast variation, proposed by Stuhrmann (Stuhrmann *et al.*, 1986; Knop *et al.*, 1986), takes advantage of the different scattering lengths of the hydrogen atoms in its spin-up and spin-down states. Normally, these two states are mixed, and the cross section of unpolarized neutrons with the undirected spins gives rise to the usual value of the scattering amplitude of hydrogen. If, however, one is able to orient the spins of a given atom, and especially hydrogen, then the interaction of *polarized* neutrons with the two different oriented states offers an important contribution to the scattering amplitude:

$$A = b + 2B\mathbf{I} \cdot \mathbf{s}, \qquad (2.6.2.5)$$

where b is the isotropic nuclear scattering amplitude, B is the spin-dependent scattering amplitude, $\mathbf{s}$ is the neutron spin, and $\mathbf{I}$ the nuclear spin. For hydrogen, $b = -0.374 \times 10^{-12}$ cm, $B = 2.9 \times 10^{-12}$ cm.

The sample protons are polarized at very low temperatures (order of mK) and high magnetic fields (several tesla) by dynamic nuclear polarization, *i.e.* by spin–spin coupling with the electron spins of a paramagnetic metallo-organic compound present in the sample, which are polarized by a resonant microwave frequency. It is clear that the principles mentioned above also apply to other than biological and chemical material.

2.6.2.4. *Long wavelengths*

An important aspect of neutron scattering is the ease of using long wavelengths: Long-wavelength X-rays are produced efficiently only by synchrotrons, and therefore their cost is similar to that of neutrons. Unlike neutrons, however, they suffer from their strong interaction with matter. This disadvantage, which is acceptable with the commonly used Cu $K\alpha$ radiation, is in most cases prohibitive for wavelengths of the order of 1 nm.

Very low Q values are more easily obtained with long wavelengths than with very small angles, as is necessary with X-rays, since the same Q value can be observed further away from the direct beam. Objects of linear dimensions of several 100 nm, *e.g.* opals, where spherical particles of amorphous silica form a close-packed lattice with cell dimensions of up to several hundreds of nm, can still be investigated easily with neutrons. X-ray double-crystal diffractometers (Bonse & Hart, 1966), which may also reach very low Q, are subject to transmission problems, and neutron DCD's again perform better.

2.6.2.5. *Sample environment*

Important new fields of low-Q scattering, such as dynamic studies of polymers in a shear gradient and time-resolved studies of samples under periodic stress or under high pressure, have become accessible by neutron scattering because the weak interaction of neutrons with (homogeneous) matter permits the use of relatively thick (several mm) sample container walls, for example of cryostats, Couette-type shearing apparatus (Lindner & Oberthür, 1985, 1988), and ovens. Air scattering is not prohibitive, and easy-to-handle standard quartz cells serve as sample containers rather than very thin ones with mica windows in the case of X-rays.

Unlike with X-rays, samples can be relatively thick, and nevertheless be studied to low Q values. This is particularly evident for metals, where X-rays are usually restricted to thin foils, but neutrons can easily accept samples 1–10 mm thick.

2.6.2.6. *Incoherent scattering*

Incoherent scattering is produced by the interaction of neutrons with nuclei that are not in a fixed phase relation with that of other nuclei. It arises, for example, when molecules do not all contain the same isotope of an element (isotopic incoherent scattering). The most important source of incoherent scattering in SANS, however, is the spin-incoherent scattering from protons. It results from the fact that only protons and neutrons with identical spin directions can form an intermediate compound nucleus. The statistical probabilities of the parallel and antiparallel spin orientations, the similarity in size of the scattering lengths for spin up and spin down and their opposite sign result in an extremely large incoherent scattering cross section for ^{1}H, together with a coherent cross section of normal magnitude (but negative sign). Incoherent scattering contributes a background that can be by orders of magnitude more important than the coherent signal, especially at larger Q. On the other hand, it can be used for the calibration of the incoming intensity and of the detector efficiency (see below).

2.6.2.6.1. *Absolute scaling*

Wignall & Bates (1987) compare many different methods of absolute calibration of SANS data. Since the scattering from a thin water sample is frequently already being used for correcting the detector response [see §2.6.2.6.2], there is an evident advantage for performing the absolute calibration by H_2O scattering.

For a purely isotropic scatterer, the intensity scattered into a detector element of surface ΔA spanning a solid angle $\delta\Omega = \Delta A / 4\pi L^2$ can be expressed as

$$\Delta I = I_0 (1 - T_i) \delta\Omega g / 4\pi, \qquad (2.6.2.6)$$

with T_i the transmission of the isotropic scatterer, *i.e.* the relation of the number of neutrons in the primary beam measured within a time interval Δt after having passed through the sample, I_{Tr}, and the number of neutrons I_0 observed within Δt without the sample. In practice, T_i is measured with an attenuated beam; typical attenuation factors are about 100 to 1000. g is a geometrical factor taking into account the sample surface and the solid angle subtended by the apparent source, *i.e.* the cross section of the neutron guide exit.

Vanadium is an incoherent scatterer frequently used for absolute scaling. Its scattering cross section, however, is more than an order of magnitude lower than that of protons. Moreover, the surface of vanadium samples has to be handled with much care in order to avoid important contributions from

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

$$I(Q) = P(Q)NT_sI_0g\left(\sum b_i - \rho_sV\right)^2, \qquad (2.6.2.7)$$

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

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

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) = $ constant $\times A_sQ^{-4}$ 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): $I(Q) \simeq I(0)\,(1 - a^2Q^2/3 + \ldots)$. 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 $g\,l^{-1}$, 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:

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

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

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

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 (2.6.2.11a) 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.

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 macro-molecular 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,

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

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

$$I(Q) = P(Q)S(Q). \qquad (2.6.2.15)$$

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).