

2.6. SMALL-ANGLE TECHNIQUES

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 Kosterz, 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 ($^1\text{H}_2\text{O}$) and heavy water ($^2\text{H}_2\text{O}$), but also with other solvents available in protonated and deuterated form (ethanol, cyclohexane, *etc.*). The scattering-length density of H_2O varies between $-0.562 \times 10^{10} \text{ cm}^{-2}$ for normal water, which is nearly pure $^1\text{H}_2\text{O}$, and $6.404 \times 10^{10} \text{ cm}^{-2}$ 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 $^1\text{H}/^2\text{H}$ 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 $^2\text{H}/(^2\text{H}+^1\text{H})$ 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 $^2\text{H}_2\text{O}$ 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

$$I(Q) = [d\sigma(Q)/d\Omega]NTA I_0 \Delta\Omega, \quad (2.6.2.1)$$

with the scattering cross section per particle and unit solid angle

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

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

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

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

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

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

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

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 infinite 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 $^1\text{H}/^2\text{H}$ 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

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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:

$$A = b + 2BI \cdot s, \quad (2.6.2.5)$$

where b is the isotropic nuclear scattering amplitude, B is the spin-dependent scattering amplitude, s is the neutron spin, and 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 ^1H , 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

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

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_T , 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