

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 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 ($^1\text{H}_2\text{O}$) 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

$$I(Q) = [d\sigma(Q)/d\Omega]NTA|_0\Delta\Omega, \quad (2.6.2.1)$$

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