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19.4. Small-angle neutron scattering

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19.4.1. Introduction

Diffuse scatter results when X-ray and neutron beams pass through gases and liquids. It is caused by local inhomogeneities, which all fluids invariably contain, at least transiently, and information about them can be obtained by analysis of the scatter they cause. This diffuse scatter is rotationally symmetric about the direction defined by the incident beam because, on average, gases and liquids are isotropic, and it does depend on scattering angle, 2θ. The intensity of the diffuse X-ray scatter of water, for example, is small at small 2θ, and it reaches a maximum at equivalent Bragg spacings equal to the reciprocal of the average oxygen–oxygen distance. In addition to a 'water ring' at high scattering angles, the diffuse scatter of macromolecular solutions includes a peak at $2\theta = 0$, due to the presence of the macromolecules themselves. If ångstrom-wavelength radiation is used, the central macromolecular peak is entirely contained in the region where $\sin(2\theta) \simeq 2\theta$. This is the region examined in small-angle scattering experiments.

Several properties of macromolecules can be determined by analysing their small-angle solution scattering, among them molecular weight, radius of gyration and maximum linear dimension. Approximate shapes can sometimes be obtained, and if a macromolecule is a complex of different chemical species, information about the distribution of its components may emerge. Hydration and conformational changes are also studied this way.

The molecular properties that can be investigated by small-angle scattering are the same for thermal neutrons and X-rays, but the advantages of neutrons are so great that if the equipment required were not so expensive, not many would do small-angle X-ray scattering (SAXS). They are all manifestations of the differences between the ways in which neutrons and X-ray photons interact with matter. For example, thermal neutrons have very low kinetic energies ($\simeq kT$), and, consequently, the energy they deposit in a sample when they are scattered inelastically is negligible. X-ray photons have large energies, and when they are absorbed or scattered inelastically, damaging amounts of energy are deposited. Thus, samples are 'safer' in neutron beams than they are in X-ray beams.

The cross section for X-ray absorption rises so fast with increasing wavelength that it is impractical to do solution-scattering experiments using X-rays with wavelengths much greater than 1.5 Å. The combination of this and the fact that X-ray beams scatter strongly off the edges of optical-track components makes it difficult to build small-angle X-ray spectrometers that measure diffuse scatter at equivalent reciprocal spacings of 0.001 Å^{-1} or less. The cross section for thermal neutron absorption is small and nearly independent of wavelength over the range 1–10 Å. Furthermore, parasitic neutron scatter is easy to control. Thus, it is comparatively straightforward to build small-angle neutron scattering (SANS) spectrometers that measure diffuse scatter at reciprocal spacings considerably less than 0.001 Å^{-1}.

Even more important to those interested in SANS are the vistas opened up by the huge difference in scattering length that exists between ^{1}H and ^{2}H (henceforth termed H and D), to which we will return below. In brief, the scatter of macromolecular solutions can be significantly altered by replacing some or all of the H atoms with D atoms. This control greatly extends the range of problems that can be addressed by SANS, and the chemical 'cost' is minimal. A perdeuterated molecule is almost identical to its protonated counterpart. The X-ray scattering of substances depends on the number of electrons they contain, and when this number is changed, chemical properties change also.

Relative to SAXS, the sole disadvantage of SANS is a phenomenon called incoherent scatter, which is a comparatively minor aspect of X-ray work. Neutrons are scattered primarily by atomic nuclei, and if a nucleus has spin, its scattering length depends on the orientation of its spin relative to that of each neutron with which it interacts. Since nuclear spins are usually unoriented in SANS samples, this spin-orientation dependence leads to a random atom-to-atom variation in scattering length. Coherent scatter, on which all diffraction effects depend, is determined by average scattering-length values. The scatter due to fluctuations about the average is incoherent, and in the low-angle region incoherent scatter manifests itself as a featureless background that is independent of scattering angle. The cross section for incoherent scattering is very large for H atoms, and since both water and biological macromolecules contain large proportions of H atoms, incoherent scatter is often a dominant source of background.

Some useful general references for small-angle scattering in general and neutron scattering in particular are Bacon (1975), Glatter & Kratky (1982) and Guinier (1955, 1962).

19.4.2. Fundamental relationships

For most purposes, a dilute macromolecular solution can be thought of as a macromolecular gas, and for that reason it is appropriate to apply Debye's theory for gas scatter to macromolecular solutions (Debye, 1915). Debye's master equation can be cast into neutron terms as follows:

$$I(Q) \propto I_0 \sum\sum b_i b_j \sin(Qr_{ij})/(Qr_{ij}), \qquad (19.4.2.1)$$

where $I(Q)$ is the amount of scattered radiation observed at Q, I_0 is the intensity of the incident beam, b_i and b_j are the scattering lengths of the ith and jth atoms in the molecule, r_{ij} is the distance between atoms i and j, and $Q = (4\pi/\lambda)\sin\theta$, λ being the wavelength of the radiation used and θ being half the scattering angle. When applied to molecules in solution, both summations must include not only all the atoms that are covalent components of the molecule in question, but also all the associated solvent atoms, because when a macromolecule dissolves, the inhomogeneity created includes the counterions associated with it, its solvation layer *etc.* Equation (19.4.2.1) holds for a single molecule; if the number of molecules contributing to scattering in some sample is N, the scattering profile measured will be N times the profile due to a single molecule. The inhomogeneities responsible for small-angle scatter have linear dimensions of the order of 10 Å or more and, hence, have volumes that contain large numbers of atoms. In addition, interatomic spacings cannot be resolved using small-angle data. Thus, it is appropriate to discuss small-angle scattering in terms of electron densities for X-rays or scattering-length densities for thermal neutrons. The scattering-length density of a volume, ρ, is given by

$$\rho \equiv \sum b_i/V, \qquad (19.4.2.2)$$

where b_i is the scattering length of the ith atom in volume V and the summation runs over all atoms in the volume.

Recasting the Debye equation in terms of scattering lengths, one obtains

$$I(Q) \propto I_0 \int\int \rho(\mathbf{r}_i)\rho(\mathbf{r}_j)[\sin(Qr_{ij})/(Qr_{ij})]\ \mathrm{d}V_i\ \mathrm{d}V_j,$$

where $\rho(\mathbf{r}_i)$ and $\rho(\mathbf{r}_j)$ are the scattering-length densities in volume elements whose positions are described by vectors $\mathbf{r}_i$ and $\mathbf{r}_j$, $r_{ij} = |\mathbf{r}_i - \mathbf{r}_j|$, and both integrals run over the volume of the entire

macromolecule plus the surrounding perturbed volume. Both this equation and equation (19.4.2.1) implicitly assume that the medium surrounding each molecule is a vacuum, which is not true for the molecules in solution. The effect of solvent on low-angle scattering can be taken care of by subtracting the average scattering length of the solvent, ρ_0, from the scattering-length densities within the molecule. Thus,

$$I(Q) \propto I_0 \int\int [\rho(\mathbf{r}_i) - \rho_0][\rho(\mathbf{r}_j) - \rho_0]_j [\sin(Qr_{ij})/(Qr_{ij})] \, \mathrm{d}V_i \, \mathrm{d}V_j. \quad (19.4.2.3)$$

The quantity $[\rho(\mathbf{r}_i) - \rho_0]$ is a *contrast*, and, as will be shown, contrast manipulation is a major component of SANS experiments.

Equation (19.4.2.3) can be evaluated a second way, because the $(\sin x)/x$ term in the integral depends only on the distances between volume elements, not on their locations in space. Thus, a function $p(r)$ can be defined as follows:

$$p(r) \equiv \int\int [\rho(\mathbf{r}_i) - \rho_0][\rho(\mathbf{r}_i + \mathbf{r}) - \rho_0] \, \mathrm{d}V_i \, \mathrm{d}V\mathbf{r},$$

where the integral in $\mathbf{r}$ runs over all $\mathbf{r}$ such that $|\mathbf{r}| = r$, and the integral in $\mathbf{r}_i$ runs over the entire molecular volume. Written in terms of $p(r)$, equation (19.4.2.3) becomes

$$I(Q) \propto I_0 \int p(r)[\sin(Qr)/(Qr)] \, \mathrm{d}r, \quad (19.4.2.4)$$

where the integral runs from $r = 0$ to $r_{\max}$, the maximum atom-to-atom length within the molecule.

Note that if contrast was constant within a macromolecule, $p(r)$ would be proportional to the distribution of interatomic distances in the molecule, and for that reason $p(r)$ is often called the *length distribution*. Note also that $p(r)$ is simply the molecule's Patterson function, rotationally averaged about its origin. Note, finally, that $p(r)$ is the summation of a large, but finite, number of sharp, discrete interatomic distance peaks, each with its own weight. If the individual interatomic peaks in this 'length spectrum' could be assigned, *i.e.*, if the atoms responsible for each one could be identified, it would be possible to determine the three-dimensional structure of the molecule in question, save for uncertainty about its hand.

Since solution-scattering profiles can be computed by sine transformations of length distributions, it is reasonable to hope that a transformation might exist that enables one to compute length distributions once solution-scattering profiles have been measured. There is (Debye & Bueche, 1949; Debye & Pirenne, 1938):

$$p(r) \propto r \int QI(Q) \sin(Qr) \, \mathrm{d}r. \quad (19.4.2.5)$$

Two practical issues must be addressed when carrying out the operation implied by equation (19.4.2.5) because the integral it contains runs from $Q = 0$ to ∞. Firstly, scattering is never measured at $Q = 0$ due to interference with the direct beam. Secondly, the largest value of Q for which $I(Q)$ is measured is always less than ∞. The absence of data at very small values of Q is easily addressed, because a soundly based method exists for extrapolating the low-angle data to $Q = 0$ (see below). The lack of data at high Q is harder to cope with, but it can be dealt with approximately using Porod's Law (Porod, 1951, 1952) and the impact of its absence on molecular parameters deduced from small-angle data is easy to estimate. In any case, it is important to realize that length distributions represent the sum total of the information that can be extracted from solution-scattering experiments.

The problem of extrapolating small-angle data to $Q = 0$ was solved by Guinier (1939). He demonstrated that, at very small angles,

$$I(Q) \propto I(0) \exp[-(QR_g)^2/3], \quad (19.4.2.6)$$

where R_g is the radius of gyration, and

$$R_g \equiv (\{\int [\rho(\mathbf{r}) - \rho_0]|\mathbf{r}|^2 \, \mathrm{d}V\}/\{\int [\rho(\mathbf{r}) - \rho_0] \, \mathrm{d}V\})^{1/2}. \quad (19.4.2.7)$$

The origin of the vector $\mathbf{r}$ in this equation is the centre of gravity of the macromolecule's scattering-length density distribution, *i.e.*, it is the point where

$$0 = \{\int [\rho(\mathbf{r}) - \rho_0]\mathbf{r} \, \mathrm{d}V\}/\{\int [\rho(\mathbf{r}) - \rho_0] \, \mathrm{d}V\}.$$

It follows from equation (19.4.2.6) that if the lowest-angle data collected are plotted in the form $\ln[I(Q)]$ versus Q^2, a straight line should result, the slope of which is $(R_g^2/3)$ and the intercept of which at $Q = 0$ is $I(0)$. Note that data have to be obtained at scattering angles well inside the region where $I(Q) \sim I(0)/2$ in order for this formula to hold; if the data are thus obtained, a radius of gyration estimate will emerge. The radius of gyration of an object is the root-mean-squared distance between its centre of gravity and the elements of which it is composed.

As might be expected, $I(0)$ and R_g can also be computed from $p(r)$. Consider the magnitude of $I(Q)$ at $Q = 0$. Since the $\sin x/x$ term in equation (19.4.2.4) is 1 at $Q = 0$,

$$I(0) \propto I_0 \int\int [\rho(\mathbf{r}_i) - \rho_0][\rho(\mathbf{r}_j) - \rho_0] \, \mathrm{d}V_i \, \mathrm{d}V_j = \int p(r) \, \mathrm{d}r. \quad (19.4.2.8)$$

Thus, $I(0)$, the forward scatter, is proportional to the integral of the length distribution. It is easy to show that R_g equals $(M/2)^{1/2}$, where M is the second moment of $p(r)$ given by

$$M = [\int r^2 p(r) \, \mathrm{d}r]/[\int p(r) \, \mathrm{d}r]. \quad (19.4.2.9)$$

The average atom-to-atom distance in a molecule, r_{ave}, is easy to compute if $p(r)$ is known from

$$r_{\mathrm{ave}} = [\int rp(r) \, \mathrm{d}r]/[\int p(r) \, \mathrm{d}r]. \quad (19.4.2.10)$$

The reason forward scatter, $I(0)$, is interesting is its dependence on molecular weight. As equation (19.4.2.8) suggests, the forward scatter measured for a sample is proportional to N times the square of the product of the average contrast between a molecule and its solvent and the molecular volume, where N is again the number of molecules contributing to the signal observed. Since average contrasts can be estimated from chemical compositions and partial specific volumes, $I(0)$ measurements can be used to estimate molecular weights. If the $I(0)$ values of solutions of a set of molecules of similar chemical composition are compared, it will be found that $I(0)$ divided by the weight concentration of each sample is proportional to molecular weight.

This procedure for estimating molecular weights can fail. Suppose $\int [\rho(\mathbf{r}_i) - \rho_0) \, \mathrm{d}V = 0$, *i.e.*, the scattering-length density of the solvent is the same as the average scattering-length density of the macromolecule. Then $I(0)$ will be zero, the solution-scattering profile will lack a peak at small angles and no molecular-weight estimate will result. Under these conditions, the macromolecule is said to be 'contrast matched'. It is easy to contrast-match biological macromolecules in the context of SANS experiments, because all biological macromolecules that have not been labelled with ^{2}H have average scattering-length densities between those of H_2O and D_2O (see below).

19.4.3. Contrast variation

19.4.3.1. *Variation of solvent density*

The principle of contrast variation was studied in early work by Bragg & Perutz (1952), who observed that the magnitudes of low-order reflections in X-ray studies of protein crystals were reduced as the salt concentration in the solvent was raised. Following their concept, the effective scattering density of a dissolved particle is

$$\rho(\mathbf{r}) = \rho(\mathbf{r})_{\mathrm{solute}} - \rho(\mathbf{r})_{\mathrm{solvent}} = \rho(\mathbf{r})_{\mathrm{solute}} - \rho_{\mathrm{solvent}},$$

references