2. DIFFRACTION GEOMETRY AND ITS PRACTICAL REALIZATION

problem and on the source used. This range is similar for X-rays and neutrons, but neutrons interact with the nuclei of the atoms whereas X-rays interact with the electrons. The scattering efficiency increases linearly with the atomic number for X-rays. The dependence is much more complicated for neutrons and does not show a systematic trend. The essential fact in neutron scattering is the pronounced difference in the scattering power between hydrogen and deuterium, which is important for varying the contrast between the particles and the matrix.

The wavelength and the scattering efficiency limit the range of small-angle scattering experiments to systems in the size range of a few nanometres up to about 100 nm. Special instruments permit the study of larger particles (Bonse & Hart, 1967; Koch, 1988). These instruments need a high intensity of the primary beam (synchrotron radiation) and are not very common.

Particles in the size range from 100 nm up to some micrometres can be investigated by static light-scattering techniques (Glatter, Hofer, Jorde & Eigner, 1985; Glatter & Hofer, 1988a,b; Hofer, Schurz & Glatter, 1989). Particles exceeding this limit can be seen in an optical microscope or can be studied with Fraunhofer diffraction (Bayvel & Jones, 1981).

Electron microscopy is in competition with all these scattering methods. It has the marked advantage of giving real pictures with rather high resolution but it has the inherent disadvantage that the preparation may introduce artefacts. Small-angle scattering, on the other hand, is a method to study macromolecules in solution, which is a very important advantage for biological samples and for polymers.

Crystallography gives more information about the particle (atomic structure) and can be applied to relatively large systems. It is possible to study particles as large as proteins and viruses if good crystals of these substances are available. The experiment needs synchrotron radiation for large molecules like proteins or viruses, *i.e.* access to a large research facility is necessary. Small-angle X-ray scattering with conventional generators is a typical next-door technique with the advantage of ready availability.

Small-angle scattering has developed into a standard measuring method during recent decades, being most powerful for the investigation of submicrometre particles.

2.6.1.2. *General principles*

In this subsection, we are concerned with X-rays only, but all equations may also be applied with slight modifications to neutron or electron diffraction. When a wave of X-rays strikes an object, every electron becomes the source of a scattered wave. All these waves have the same intensity given by the Thomson formula

$$I_e(\theta) = I_p T_f \frac{1}{a^2} \frac{1 + \cos^2 2\theta}{2}, \qquad (2.6.1.1)$$

where I_p is the primary intensity and a the distance from the object to the detector. The factor T_f is the square of the classical electron radius $(e^2/mc^2 = 7.90 \times 10^{-26} [\text{cm}^2])$. The scattering angle 2θ is the angle between the primary beam and the scattered beam. The last term in (2.6.1.1) is the polarization factor and is practically equal to 1 for all problems dealt with in this subsection. I_e should appear in all following equations but will be omitted, *i.e.* the amplitude of the wave scattered by an electron will be taken to be of magnitude 1. I_e is only needed in cases where the absolute intensity is of interest.

The amplitudes differ only by their phases φ, which depend on the positions of the electrons in space. Incoherent (Compton) scattering can be neglected for small-angle X-ray scattering. The

phase φ is $2\pi/\lambda$ times the difference between the optical path length of the wave and an arbitrary reference wave (with λ being the wavelength). The direction of the incident beam is defined by the unit vector $\mathbf{s}_0$ and of the scattered beam by $\mathbf{s}$. The angle between these two unit vectors (scattering angle) is 2θ. The path difference between the rays through a point P and an arbitrary origin O is $-\mathbf{r}(\mathbf{s} - \mathbf{s}_0)$. The phase is $\varphi = -\mathbf{h} \cdot \mathbf{r}$ if we define the scattering vector $\mathbf{h}$ as

$$\mathbf{h} = (2\pi/\lambda)(\mathbf{s} - \mathbf{s}_0). \qquad (2.6.1.2)$$

This vector bisects the angle between the scattered beam and the incident beam and has length $h = (4\pi/\lambda)\sin\theta$. We keep in mind that $\sin\theta$ may be replaced by θ in small-angle scattering. We now introduce the electron density $\rho(\mathbf{r})$. This is the number of electrons per unit volume at the position $\mathbf{r}$. A volume element dV at $\mathbf{r}$ contains $\rho(\mathbf{r})\,dV$ electrons. The scattering amplitude of the whole irradiated volume V is given by

$$A(\mathbf{h}) = \iiint \rho(\mathbf{r}) \exp(-i\mathbf{h} \cdot \mathbf{r})\,dV. \qquad (2.6.1.3)$$

We see that the amplitude A is the Fourier transform of the electron-density distribution ρ. The intensity $I(\mathbf{h})$ of the complex amplitude $A(\mathbf{h})$ is the absolute square given by the product of the amplitude and its complex conjugate A^*,

$$I(\mathbf{h}) = A(\mathbf{h})A(\mathbf{h})^* = \iiint \widetilde{\rho}^2(\mathbf{r}) \exp(-i\mathbf{h} \cdot \mathbf{r})\,dV, \qquad (2.6.1.4)$$

where $\widetilde{\rho}^2(\mathbf{r})$ is the convolution square (Bracewell, 1986):

$$\widetilde{\rho}^2(\mathbf{r}) = \iiint \rho(\mathbf{r}_1)\rho(\mathbf{r}_1 - \mathbf{r})\,dV_1. \qquad (2.6.1.5)$$

The intensity distribution in $\mathbf{h}$ or reciprocal space is uniquely determined by the structure in real space.

Until now, we have discussed the scattering process of a particle in fixed orientation in vacuum. In most cases of small-angle scattering, the following situation is present:

–The scatterers (particles or inhomogeneities) are statistically isotropic and no long-range order exists, *i.e.* there is no correlation between points at great spatial distance.

–The scatterers are embedded in a matrix. The matrix is considered to be a homogeneous medium with the electron density ρ_0. This situation holds for particles in solution or for inhomogeneities in a solid. The electron density in equations (2.6.1.3)–(2.6.1.5) should be replaced by the difference in electron density $\Delta\rho = \rho - \rho_0$, which can take positive and negative values.

The average over all orientations $\langle\ \rangle$ leads to

$$\langle \exp(-i\mathbf{h} \cdot \mathbf{r}) \rangle = \frac{\sin hr}{hr} \qquad (2.6.1.6)$$

(Debye, 1915) and (2.6.1.4) reduces to the form

$$I(h) = 4\pi \int_0^\infty r^2 \Delta\widetilde{\rho}^2(r) \frac{\sin hr}{hr}\,dr \qquad (2.6.1.7)$$

or, with

$$p(r) = r^2 \Delta\widetilde{\rho}^2(r) = r^2 V\gamma(r), \qquad (2.6.1.8)$$

to

$$I(h) = 4\pi \int_0^\infty p(r) \frac{\sin hr}{hr}\,dr; \qquad (2.6.1.9)$$

γ is the so-called correlation function (Debye & Bueche, 1949), or characteristic function (Porod, 1951). The function $p(r)$ is the so-called pair-distance distribution function PDDF (Guinier &

Fournet, 1955; Glatter, 1979). The inverse transform to (2.6.1.9) is given by

$$p(r) = \frac{1}{2\pi^2} \int\limits_0^\infty I(h)hr \sin(hr)\, \mathrm{d}h \qquad (2.6.1.10)$$

or by

$$V\gamma(r) = \frac{1}{2\pi^2} \int\limits_0^\infty I(h)h^2 \frac{\sin hr}{hr}\, \mathrm{d}h. \qquad (2.6.1.11)$$

The function $p(r)$ is directly connected with the measurable scattering intensity and is very important for the solution of the inverse scattering problem. Before working out details, we should first discuss equations (2.6.1.9) and (2.6.1.10).

The PDDF can be defined as follows: the function $p(r)$ gives the number of difference electron pairs with a mutual distance between r and $r + \mathrm{d}r$ within the particle. For homogeneous particles (constant electron density), this function has a simple and clear geometrical definition.

Let us subdivide the particle into a very large number of identical small volume elements. The function $p(r)$ is proportional to the number of lines with a length between r and $r + \mathrm{d}r$ which are found in the combination of any volume element i with any other volume element k of the particle (see Fig. 2.6.1.1). For $r = 0$, there is no other volume element, so $p(r)$ must be zero, increasing with r^2 as the number of possible neighbouring volume elements is proportional to the surface of a sphere with radius r. Starting from an arbitrary point in the particle, there is a certain probability that the surface will be reached within the distance r. This will cause the $p(r)$ function to drop below the r^2 parabola and finally the PDDF will be zero for all $r > D$, where D is the maximum dimension of the particle. So $p(r)$ is a distance histogram of the particle. There is no information about the orientation of these lines in $p(r)$, because of the spatial averaging.

In the case of inhomogeneous particles, we have to weight each line by the product of the difference in electron density $\Delta\rho$, and the differential volume element, $\mathrm{d}V$. This can lead to negative contributions to the PDDF.

We can see from equation (2.6.1.9) that every distance r gives a $\sin(hr)/(hr)$ contribution with the weight $p(r)$ to the total scattering intensity. $I(h)$ and $p(r)$ contain the same information, but in most cases it is easier to analyse in terms of distances than in terms of $\sin(x)/x$ contributions. The PDDF could be computed exactly with equation (2.6.1.10) if $I(h)$ were known for the whole reciprocal space.
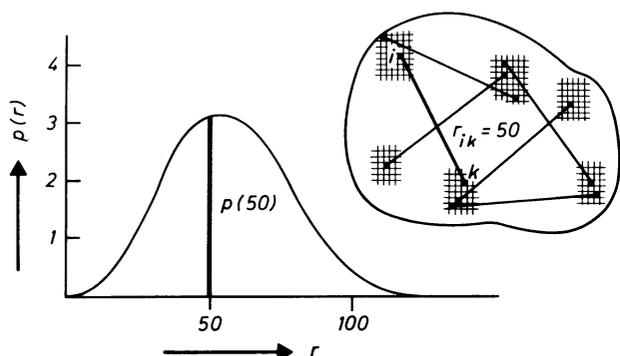
For $h = 0$, we obtain from equation (2.6.1.9)

Fig. 2.6.1.1. The height of the $p(r)$ function for a certain value of r is proportional to the number of lines with a length between r and $r + \mathrm{d}r$ within the particle.

$$I(0) = I(\overline{\Delta\rho})^2 V^2 = 4\pi \int\limits_0^\infty p(r)\, \mathrm{d}r, \qquad (2.6.1.12)$$

i.e. the scattering intensity at h equal to zero is proportional to the area under the PDDF. From equation (2.6.1.11), we find

$$V\gamma(0) = \frac{1}{2\pi^2} \int I(h)h^2\, \mathrm{d}h = V\overline{\Delta\rho^2} \qquad (2.6.1.13)$$

(Porod, 1982), *i.e.* the integral of the intensity times h^2 is related to the mean-square fluctuation of the electron density irrespective of the structure. We may modify the shape of a particle, the scattering function $I(h)$ might be altered considerably, but the integral (2.6.1.13) must remain invariant (Porod, 1951).

$$\text{Invariant } Q = \int\limits_0^\infty I(h)h^2\, \mathrm{d}h. \qquad (2.6.1.14)$$

2.6.1.3. *Monodisperse systems*

In this subsection, we discuss scattering from monodisperse systems, *i.e.* all particles in the scattering volume have the same size, shape, and internal structure. These conditions are usually met by biological macromolecules in solution. Furthermore, we assume that these solutions are at infinite dilution, which is taken into account by measuring a series of scattering functions at different concentrations and by extrapolating these data to zero concentration. We continue with the notations defined in the previous subsection, which coincide to a large extent with the notations in the original papers and in the textbooks (Guinier & Fournet, 1955; Glatter & Kratky, 1982). There is a notation created by Luzzati (1960) that is quite different in many details. A comparison of the two notations is given in the Appendix of Pilz, Glatter & Kratky (1980).

The particles can be roughly described by some parameters that can be extracted from the scattering function. More information about the shape and structure of the particles can be found by detailed discussion of the scattering functions. At first, this discussion will be about homogeneous particles and will be followed by some aspects for inhomogeneous systems. Finally, we have to discuss the influence of finite concentrations on our results.

2.6.1.3.1. *Parameters of a particle*

Total scattering length. The scattering intensity at $h = 0$ must be equal to the square of the number of excess electrons, as follows from equations (2.6.1.7) and (2.6.1.12):

$$I(0) = (\Delta\rho)^2 V^2 = 4\pi \int\limits_0^\infty p(r)\, \mathrm{d}r. \qquad (2.6.1.15)$$

This value is important for the determination of the molecular weight if we perform our experiments on an absolute scale (see below).

Radius of gyration. The electronic radius of gyration of the whole particle is defined in analogy to the radius of gyration in mechanics:

$$R_g^2 = \frac{\int\limits_V \rho(r_i)r_i^2\, \mathrm{d}V_i}{\int\limits_V \rho(r_i)\, \mathrm{d}V_i}. \qquad (2.6.1.16)$$

It can be obtained from the PDDF by