

## 2.6. SMALL-ANGLE TECHNIQUES

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

$$p(r) = \frac{1}{2\pi^2} \int_0^\infty I(h)hr \sin(hr) dh \quad (2.6.1.10)$$

or by

$$V\gamma(r) = \frac{1}{2\pi^2} \int_0^\infty I(h)h^2 \frac{\sin hr}{hr} dh. \quad (2.6.1.11)$$

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  $r + dr$  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  $r + dr$  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,  $dV$ . 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)

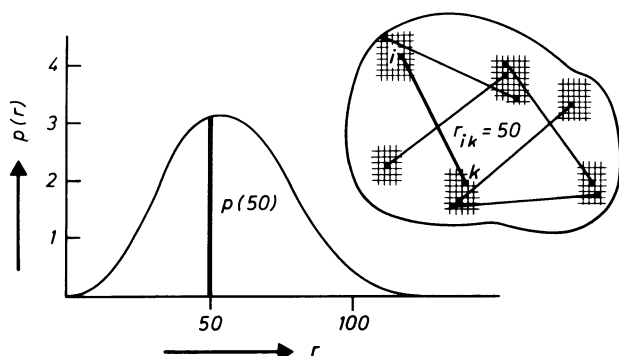


Fig. 2.6.1.1. The height of the  $p(r)$  function for a certain value of  $r$  is proportional to the number of lines with a length between  $r$  and  $r + dr$  within the particle.

$$I(0) = I(\overline{\Delta\rho})^2 V^2 = 4\pi \int_0^\infty p(r) dr, \quad (2.6.1.12)$$

*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

$$V\gamma(0) = \frac{1}{2\pi^2} \int_0^\infty I(h)h^2 dh = V\overline{\Delta\rho^2} \quad (2.6.1.13)$$

(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).

$$\text{Invariant } Q = \int_0^\infty I(h)h^2 dh. \quad (2.6.1.14)$$

## 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):

$$I(0) = (\Delta\rho)^2 V^2 = 4\pi \int_0^\infty p(r) dr. \quad (2.6.1.15)$$

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:

$$R_g^2 = \frac{\int_V \rho(r_i)r_i^2 dV_i}{\int_V \rho(r_i) dV_i}. \quad (2.6.1.16)$$

It can be obtained from the PDDF by

## 2. DIFFRACTION GEOMETRY AND ITS PRACTICAL REALIZATION

Table 2.6.1.1. *Formulae for the various parameters for h (left) and m (right) scales*

$R = K\sqrt{\tan \alpha}$ $\tan \alpha = \frac{\Delta \log I(h)}{\Delta h^2}$ $K = \sqrt{\frac{3}{\log e}} = 2.628$	$R = K \frac{\lambda a}{2\pi} \sqrt{\tan \alpha}$ $\tan \alpha = \frac{\Delta \log I(m)}{\Delta m^2}$
$R_c = K_c \sqrt{\tan \alpha}$ $\tan \alpha = \frac{\Delta \log [I(h)h]}{\Delta h^2}$ $K_c = \sqrt{\frac{2}{\log e}} = 2.146$	$R_c = K_c \frac{\lambda a}{2\pi} \sqrt{\tan \alpha}$ $\tan \alpha = \frac{\Delta \log [I(m)m]}{\Delta m^2}$
$R_t = K_t \sqrt{\tan \alpha}$ $\tan \alpha = \frac{\Delta \log [I(h)h^2]}{\Delta h^2}$ $K_t = \sqrt{\frac{1}{\log e}} = 1.517$	$R_t = K_t \frac{\lambda a}{2\pi} \sqrt{\tan \alpha}$ $\tan \alpha = \frac{\Delta \log [I(m)m^2]}{\Delta m^2}$
$V = 2\pi^2 \frac{I(0)}{Q}$ $Q = \int I(h)h^2 dh$	$V = \frac{\lambda^3 a^3}{4\pi} \frac{I(0)}{Q_m}$ $Q_m = \int I(m)m^2 dm$
$A = 2\pi \frac{[I(h)h]_0}{Q}$	$A = \frac{\lambda^2 a^2}{2\pi} \frac{[I(m)m]_0}{Q_m}$
$T = \pi \frac{[I(h)h^2]_0}{Q}$	$T = \frac{\lambda a}{2} \frac{[I(m)m^2]_0}{Q_m}$
$M = \frac{I(0)}{P} K \frac{a^2}{cd(\Delta z)^2}$	$K = \frac{1}{I_e N_L} = 21.0$
$M_c = \frac{[I(h)h]_0}{P} \frac{K}{\pi} \frac{a^2}{cd(\Delta z)^2}$	$M_c = \frac{[I(m)m]_0}{P} \frac{2K}{\lambda} \frac{a}{cd(\Delta z)^2}$
$M_t = \frac{[I(h)h^2]_0}{P} \frac{K}{2\pi} \frac{a^2}{cd(\Delta z)^2}$	$M_t = \frac{[I(m)m^2]_0}{P} \frac{2\pi K}{\lambda^2} \frac{1}{cd(\Delta z)^2}$
$\overline{(\Delta \rho)^2} = \frac{Q}{P} \frac{a^2}{2\pi^2 d} K$ $K = 10^{24}/I_e$ $(10^{24} = [\text{cm}/\text{\AA}]^3)$	$\overline{(\Delta \rho)^2} = \frac{Q_m}{P} \frac{4\pi}{\lambda^3 ad} K$
$O_s = \pi \frac{K}{Q}$ $K = \lim_{h \rightarrow \infty} I(h)h^4$	$O_s = \frac{2\pi^2}{\lambda a} \frac{K_m}{Q_m}$ $K = \lim_{m \rightarrow \infty} I(m)m^4$

$$R_g^2 = \frac{\int_0^\infty p(r)r^2 dr}{2 \int_0^\infty p(r) dr} \quad (2.6.1.17)$$

or from the innermost part of the scattering curve [Guinier approximation (Guinier, 1939)]:

$$I(h) = I(0) \exp(-h^2 R_g^2/3). \quad (2.6.1.18)$$

A plot of  $\log[I(h)]$  vs  $h^2$  (Guinier plot) shows at its innermost part a linear descent with a slope  $\tan \alpha$ , where

$$R_g = K\sqrt{\tan \alpha}$$

(see Table 2.6.1.1).

The radius of gyration is related to the geometrical parameters of simple homogeneous triaxial bodies as follows (Mittelbach, 1964):

sphere (radius $R$ )	$R_g^2 = (3/5)R^2$
hollow sphere (radii $R_1$ and $R_2$ )	$R_g^2 = (3/5) \frac{R_2^5 - R_1^5}{R_2^3 - R_1^3}$
ellipsoid (semi-axes $a, b, c$ )	$R_g^2 = (1/5)(a^2 + b^2 + c^2)$
parallelepiped (edge lengths $A, B, C$ )	$R_g^2 = (1/12)(A^2 + B^2 + C^2)$
elliptic cylinder (semi-axes $a, b$ ; height $h$ )	$R_g^2 = \frac{a^2 + b^2}{4} + \frac{h^2}{12} = R_c^2 + \frac{h^2}{12}$
hollow cylinder (height $h$ and radii $r_1, r_2$ )	$R_g^2 = \frac{r_1^2 + r_2^2}{2} + \frac{h^2}{12}$

*Radius of gyration of the cross section.* In the special case of rod-like particles, the two-dimensional analogue of  $R_g$  is called radius of gyration of the cross section  $R_c$ . It can be obtained from

$$R_c^2 = \frac{\int_0^\infty p_c(r)r^2 dr}{2 \int_0^\infty p_c(r) dr}, \quad (2.6.1.19)$$

where  $p_c(r)$  is the PDDF of the cross section or it can be calculated from the innermost part of the scattering intensity of the cross section  $I_c(h)$ :

$$I_c(h) = I_c(0) \exp(-h^2 R_c^2/2), \quad (2.6.1.20)$$

with  $I_c(h) = I(h)h$  (see Table 2.6.1.1).

*Radius of gyration of the thickness.* A similar definition exists for lamellar particles. The one-dimensional radius of gyration of the thickness  $R_t$  can be calculated from

$$R_t^2 = \frac{\int_0^\infty p_t(r)r^2 dr}{2 \int_0^\infty p_t(r) dr}, \quad (2.6.1.21)$$

or from the innermost part of the scattered intensity of thickness  $I_t(h)$ :

$$I_t(h) = I_t(0) \exp(-h^2 R_t^2), \quad (2.6.1.22)$$

with  $I_t(h) = I(h)h^2$  (see Table 2.6.1.1 and §2.6.1.3.2.1).

*Volume.* The volume of a homogeneous particle is given by

$$V = 2\pi^2 \frac{I(0)}{Q}. \quad (2.6.1.23)$$

This equation follows from equations (2.6.1.12)–(2.6.1.14). Such volume determinations are subject to errors as they rely on the validity of an extrapolation to zero angle [to obtain  $I(0)$ ] and to larger angles ( $h^{-4}$  extrapolation for  $Q$ ). Scattering functions cannot be measured from  $h$  equal to zero to  $h$  equal to infinity.

## 2.6. SMALL-ANGLE TECHNIQUES

*Surface.* The surface  $S$  of one particle is correlated with the scattering intensity  $I_1(h)$  of this particle by

$$I_1(h)|_{h \rightarrow \infty} = (\Delta\rho)^2 \frac{2\pi}{h^4} S. \quad (2.6.1.24)$$

Determination of the absolute intensity can be avoided if we calculate the specific surface  $O_s$  (Mittelbach & Porod, 1965)

$$O_s = S/V = \pi \frac{\lim_{h \rightarrow \infty} [I(h)h^4]}{Q}. \quad (2.6.1.25)$$

*Cross section, thickness, and correlation length.* By similar equations, we can find the area  $A$  of the cross section of a rod-like particle

$$A = 2\pi \frac{[I(h)h]_{h \rightarrow 0}}{Q} \quad (2.6.1.26)$$

and the thickness  $T$  of lamellar particles by

$$T = \pi \frac{[I(h)h^2]_{h \rightarrow 0}}{Q} \quad (2.6.1.27)$$

but the experimental accuracy of the limiting values  $[I(h)h]_{h \rightarrow 0}$  and  $[I(h)h^2]_{h \rightarrow 0}$  is usually not very high.

The correlation length  $l_c$  is the mean width of the correlation function  $\gamma(r)$  (Porod, 1982) and is given by

$$l_c = \frac{\pi}{Q} \int_0^{\infty} I(h)h \, dh. \quad (2.6.1.28)$$

The *maximum dimension*  $D$  of a particle would be another important particle parameter, but it cannot be calculated directly from the scattering function and will be discussed later.

*Persistence length*  $a_p$ . An important model for polymers in solution is the so-called worm-like chain (Porod, 1949; Kratky & Porod, 1949). The degree of coiling can be characterized by the persistence length  $a_p$  (Kratky, 1982b). Under the assumption that the persistence length is much larger than the cross section of the polymer, it is possible to find a transition point  $h^+$  in an  $I(h)h^2$  vs  $h$  plot where the function starts to be proportional to  $h$ . There is an approximation

$$h^+ a_p \simeq 2.3, \quad (2.6.1.29)$$

depending on the length of the chain (Heine, Kratky & Roppert, 1962). For further details, see Kratky (1982b).

*Molecular weight. Particles of arbitrary shape.* The particle is measured at high dilution in a homogeneous solution and has an isopotential specific volume  $v_2'$  and  $z_2$  mol. electrons per gram, *i.e.* the molecule contains  $z_2 M$  electrons if  $M$  is the molecular weight. The number of effective mol. electrons per gram is given by

$$\Delta z_2 = (z_2 - v_2' \rho_0), \quad (2.6.1.30)$$

where  $\rho_0$  is the mean electron density of the solvent. The molecular weight can be determined from the intensity at zero angle  $I(0)$ :

$$\begin{aligned} M &= \frac{I(0)}{P} \frac{a^2}{\Delta z_2^2 d c I_e N_L} \\ &= \frac{I(0)}{P} \frac{21.0 a^2}{\Delta z_2^2 d c} \end{aligned} \quad (2.6.1.31)$$

(Kratky, Porod & Kahovec, 1951), where  $P$  is the total intensity per unit time irradiating the sample,  $a$  [cm] is the distance between the sample and the plane of registration,  $d$  [cm] is the

thickness of the sample,  $c$  [g cm<sup>-3</sup>] is the concentration, and  $N_L$  is Loschmidt's (Avogadro's) number.

*Rod-like particles.* The mass per unit length  $M_c = M/L$ , *i.e.* the mass related to the cross section of a rod-like particle with length  $L$ , is given by a similar equation (Kratky & Porod, 1953):

$$\begin{aligned} M_c &= \frac{[I(h)h]_{h \rightarrow 0}}{P} \frac{a^2}{\pi \Delta z_2^2 d c I_e N_L} \\ &= \frac{[I(h)h]_{h \rightarrow 0}}{P} \frac{6.68 a^2}{\Delta z_2^2 d c}. \end{aligned} \quad (2.6.1.32)$$

*Flat particles.* A similar equation holds for the mass per unit area  $M_t = M/A$ :

$$\begin{aligned} M_t &= \frac{[I(h)h^2]_{h \rightarrow 0}}{P} \frac{a^2}{2\pi \Delta z_2^2 d c I_e N_L} \\ &= \frac{[I(h)h^2]_{h \rightarrow 0}}{P} \frac{3.34 a^2}{\Delta z_2^2 d c}. \end{aligned} \quad (2.6.1.33)$$

*Abscissa scaling.* The various molecular parameters can be evaluated from scattered intensities with different abscissa scaling. The abscissa used in theoretical work is  $h = (4\pi/\lambda) \sin \theta$ . The most important experimental scale is  $m$  [cm], the distance of the detector from the centre of the primary beam with the distance  $a$  [cm] between the sample and the detector plane.

$$h[\text{nm}^{-1}] = T_{hm}[\text{cm}^{-1} \text{nm}^{-1}] m[\text{cm}], \quad (2.6.1.34)$$

with

$$T_{hm} = 2\pi/\lambda a. \quad (2.6.1.35)$$

The angular scale  $2\theta$  with

$$2\theta \simeq m/a = (\lambda/2\pi)h \quad (2.6.1.36)$$

was used in the early years of small-angle X-ray scattering experiments. The formulae for the various parameters for  $m$  and the  $h$  scale can be found in Table 2.6.1.1, the formulae for the  $2\theta$  scale can be found in Glatter & Kratky (1982, p. 158).

### 2.6.1.3.2. Shape and structure of particles

In this subsection, we have to discuss how shape, size, and structure of the scattering particle are reflected in the scattering function  $I(h)$  and in the PDDF  $p(r)$ . In general, it is easier to discuss features of the PDDF, but some characteristics like symmetry give more pronounced effects in reciprocal space.

#### 2.6.1.3.2.1. Homogeneous particles

*Globular particles.* Only a few scattering problems can be solved analytically. The most trivial shape is a sphere. Here we have analytical expressions for the scattering intensity

$$I(h) = \left( 3 \frac{\sin(hR) - hR \cos(hR)}{(hR)^3} \right)^2 \quad (2.6.1.37)$$

and for the PDDF (Porod, 1948)

$$p(r) = 12x^2(2 - 3x + x^3) \quad x = r/(2R) \leq 1, \quad (2.6.1.38)$$

where  $R$  is the radius of the sphere. The graphical representation of scattering functions is usually made with a semi-log plot [ $\log I(h)$  vs  $h$ ] or with a log-log plot [ $\log I(h)$  vs  $\log h$ ]; the PDDF is shown in a linear plot. In order to compare functions from particles of different shape, it is preferable to keep the scattering intensity at zero angle (area under PDDF) and the radius of