

2. DIFFRACTION GEOMETRY AND ITS PRACTICAL REALIZATION

Composite structures – aggregates, subunits. The formation of dimers can be analysed qualitatively with the $p(r)$ function (Glatter, 1979). For an approximate analysis, it is only necessary to know the PDDF of the monomer. Different types of aggregates will have distinct differences in their PDDF. Higher aggregates generally cannot be classified unambiguously. Additional information from other sources, such as the occurrence of symmetry, can simplify the problem.

Particles that consist of aggregates of a relatively large number of identical subunits show, at low resolution, the overall structure of the whole particle. At larger angles (higher resolution), the influence of the individual subunits can be seen. In the special case of globular subunits, it is possible to determine the size of the subunits from the position of the minima of the corresponding shape factors using equation (2.6.1.39) (Glatter, 1972; Pilz, Glatter, Kratky & Moring-Claesson, 1972).

 2.6.1.3.2.2. *Hollow and inhomogeneous particles*

We have learned to classify homogeneous particles in the previous part of this section. It is possible to see from scattering data [$I(h)$ or $p(r)$] whether a particle is globular or elongated, flat or rod-like, etc., but it is impossible to determine uniquely a complicated shape with many parameters. If we allow internal inhomogeneities, we make things more complicated and it is clear that it is impossible to obtain a unique reconstruction of an inhomogeneous three-dimensional structure from its scattering function without additional *a priori* information. We restrict our considerations to special cases that are important in practical applications and that allow at least a solution in terms of a first-order approximation. In addition, we have to remember that the $p(r)$ function is weighted by the number of excess electrons that can be negative. Therefore, a minimum in the PDDF can be caused by a small number of distances, or by the addition of positive and negative contributions.

Spherically symmetric particles. In this case, it is possible to describe the particle by a one-dimensional radial excess density function $\Delta\rho(r)$. For convenience, we omit the Δ sign for excess in the following. As we do not have any angle-dependent terms, we have no loss of information from the averaging over angle. The scattering amplitude is simply the Fourier transform of the radial distribution:

$$A(h) = 4\pi \int_0^{\infty} r\rho(r) \frac{\sin(hr)}{h} dr \quad (2.6.1.51)$$

$[I(h) - A(h)^2]$ and

$$\rho(r) = \frac{1}{2\pi^2} \int_0^{\infty} hA(h) \frac{\sin(hr)}{r} dh \quad (2.6.1.52)$$

(Glatter, 1977a). These equations would allow direct analysis if $A(h)$ could be measured, but we can measure only $I(h)$. $\rho(r)$ can be calculated from $I(h)$ using equation (2.6.1.10) remembering that this function is the convolution square of $\rho(r)$ [equations (2.6.1.5) and (2.6.1.8)]. Using a *convolution square-root* technique, we can calculate $\rho(r)$ from $I(h)$ via the PDDF without having a ‘phase problem’ like that in crystallography; *i.e.* it is not necessary to calculate scattering amplitudes and phases (Glatter, 1981; Glatter & Hainisch, 1984; Glatter, 1988). This can be done because $p(r)$ differs from zero only in the limited range $0 < r < D$ (Hosemann & Bagchi, 1952, 1962). In mathematical terms, it is again the difference between a Fourier series and a Fourier integral.

Details of the technique cannot be discussed here, but it is a fact that we can calculate the radial distribution $\rho(r)$ from the scattering data assuming that the spherical scatterer is only of finite size. The hollow sphere can be treated either as a homogeneous particle with a special shape or as an inhomogeneous particle with spherical symmetry with a step function as radial distribution. The scattering function and the PDDF of a hollow sphere can be calculated analytically. The $p(r)$ of a hollow sphere has a triangular shape and the function $f(r) = p(r)/r$ shows a horizontal plateau (Glatter, 1982b).

Rod-like particles. Radial inhomogeneity. If we assume radial inhomogeneity of a circular cylinder, *i.e.* ρ is a function of the radius r but not of the angle φ or of the value of z in cylindrical coordinates, we can determine some structural details. We define $\bar{\rho}_c$ as the average excess electron density in the cross section. Then we obtain a PDDF with a linear part for $r > d$ and we have to replace $\Delta\rho$ in equation (2.6.1.46) by $\bar{\rho}_c$ with the maximum dimension of the cross section d . The $p(r)$ function differs from that of a homogeneous cylinder with the same $\bar{\rho}_c$ only in the range $0 < r \leq d$. A typical example is shown in Fig. 2.6.1.8. The functions for a homogeneous, a hollow, and an inhomogeneous cylinder with varying density $\rho_c(r)$ are shown.

Rod-like particles. Axial inhomogeneity. This is another special case for rod-like particles, *i.e.* the density is a function of the z coordinate. In Fig. 2.6.1.9, we compare two cylinders with the same size and diameter. One is a homogeneous cylinder with density $\bar{\rho}$, diameter $d = 48$ and length $L = 480$, and the other is an inhomogeneous cylinder of the same size and mean density $\bar{\rho}$, but this cylinder is made from slices with a thickness of 20 and alternating densities of $1.5\bar{\rho}$ and $0.5\bar{\rho}$, respectively. The PDDF of the inhomogeneous cylinder has ripples with the periodicity of 40 in the whole linear range. This periodicity leads to reflections in reciprocal space (first and third order in the h range of the figure).

Flat particles. Cross-sectional inhomogeneity. Lamellar particles with varying electron density perpendicular to the basal plane, where ρ is a function of the distance x from the central plane, show differences from a homogeneous lamella of the same size in the PDDF in the range $0 < r < T$, where T is the

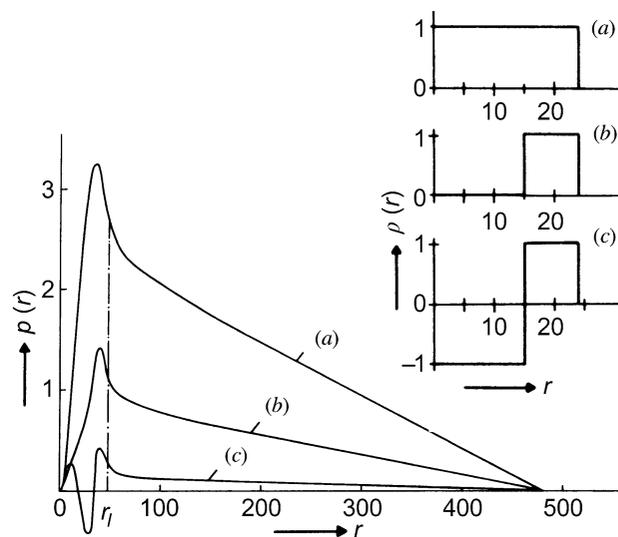


Fig. 2.6.1.8. Circular cylinder with a constant length of 480 Å and an outer diameter of 48 Å. (a) Homogeneous cylinder, (b) hollow cylinder, (c) inhomogeneous cylinder. The $p(r)$ functions are shown on the left, the corresponding electron-density distributions $\rho(r)$ on the right.

2.6. SMALL-ANGLE TECHNIQUES

thickness of the lamella. An example is given in Fig. 2.6.1.10 where we compare a homogeneous lamellar particle (with $\rho = +\frac{1}{3}$) with an inhomogeneous one, $\rho_r(x)$ being a three-step function alternating between the values $+1, -1, +1$.

Flat particles. In-plane inhomogeneity. Lamellae with a homogeneous cross section but inhomogeneities along the basal plane have a PDDF that deviates from that of a homogeneous lamella in the whole range $0 < r < D$. These deviations are a measure of the in-plane inhomogeneities; a general evaluation method does not exist. Even more complicated is the situation that occurs in membranes: these have a pronounced cross-sectional structure with additional in-plane inhomogeneities caused by the membrane proteins (Laggner, 1982; Sadler & Worcester, 1982).

Contrast variation and labelling. An important method for studying inhomogeneous particles is the method of contrast variation (Stuhrmann, 1982). By changing the contrast of the solvent, we can obtain additional information about the inhomogeneities in the particles. This variation of the contrast is much easier for neutron scattering than for X-ray scattering because hydrogen and deuterium have significantly different scattering cross sections. This technique will therefore be discussed in the section on neutron small-angle scattering.

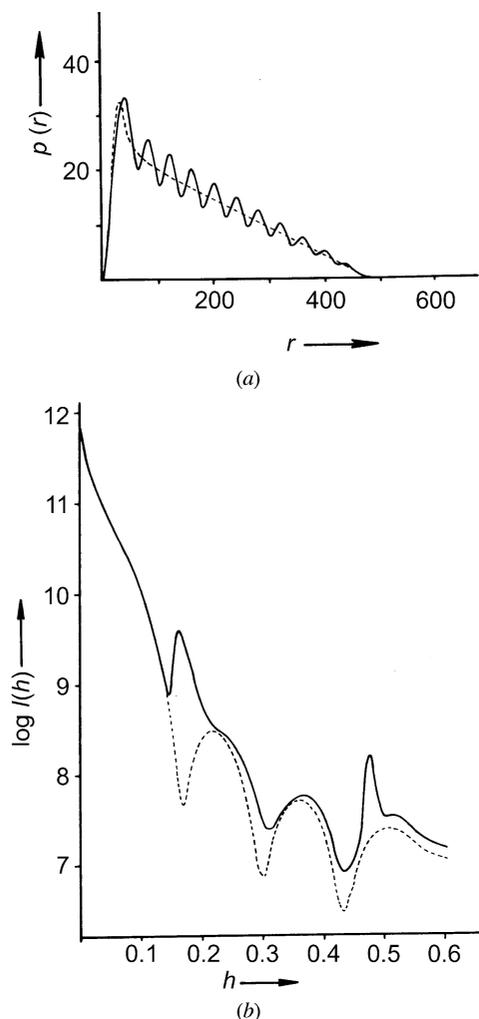


Fig. 2.6.1.9. Inhomogeneous circular cylinder with periodical changes of the electron density along the cylinder axis compared with a homogeneous cylinder with the same mean electron density. (a) $p(r)$ function; (b) scattering intensity; — inhomogeneous cylinder; - - - homogeneous cylinder.

A method for distance determination with X-rays by heavy-atom labelling was developed by Kratky & Worthman (1947). These ideas are now used for the determination of distances between deuterated subunits of complex macromolecular structures with neutron scattering.

High-resolution experiments. A special type of study is the comparison of the structures of the same molecule in the crystal and in solution. This is done to investigate the influence of the crystal field on the polymer structure (Krigbaum & Kügler, 1970; Damaschun, Damaschun, Müller, Ruckpaul & Zinke, 1974; Heidorn & Trehwella, 1988) or to investigate structural changes (Ruckpaul, Damaschun, Damaschun, Dimitrov, Jänig, Müller, Pürschel & Behlke, 1973; Hubbard, Hodgson & Doniach, 1988). Sometimes such investigations are used to verify biopolymer structures predicted by methods of theoretical physics (Müller, Damaschun, Damaschun, Misselwitz, Zirwer & Nothnagel, 1984). In all cases, it is necessary to measure the small-angle scattering curves up to relatively high scattering angles ($h \approx 30 \text{ nm}^{-1}$, and more). Techniques for such experiments have been developed during recent years (Damaschun, Gernat, Damaschun, Bychkova & Ptitsyn, 1986; Gernat, Damaschun, Kröber, Bychkova & Ptitsyn, 1986; I'anson, Bacon, Lambert, Miles, Morris, Wright & Nave, 1987) and need special evaluation methods (Müller, Damaschun & Schrauber, 1990).

2.6.1.3.3. Interparticle interference, concentration effects

So far, only the scattering of single particles has been treated, though, of course, a great number of these are always present. It has been assumed that the intensities simply add to give the total diffraction pattern. This is true for a very dilute solution, but with increasing concentration interference effects will contribute. Biological samples often require higher concentrations for a sufficient signal strength. We can treat this problem in two different ways:

-We accept the interference terms as additional information about our system under investigation, thus observing the spatial arrangement of the particles.

-We treat the interference effect as a perturbation of our single-particle concept and discuss how to remove it.

The first point of view is the more general, but there are many open questions left. For many practical applications, the second point of view is important.

The radial distribution function. In order to find a general description, we have to restrict ourselves to an isotropic assembly of monodisperse spheres. This makes it possible to

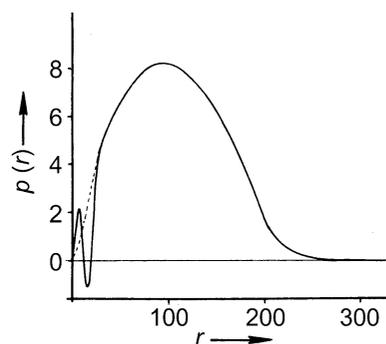


Fig. 2.6.1.10. $p(r)$ function of a lamellar particle. The full line corresponds to an inhomogeneous particle, $\rho_r(x)$ is a three-step function with the values $+1, -1, +1$. The broken line represents the homogeneous lamella with $\rho = +\frac{1}{3}$.