

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.

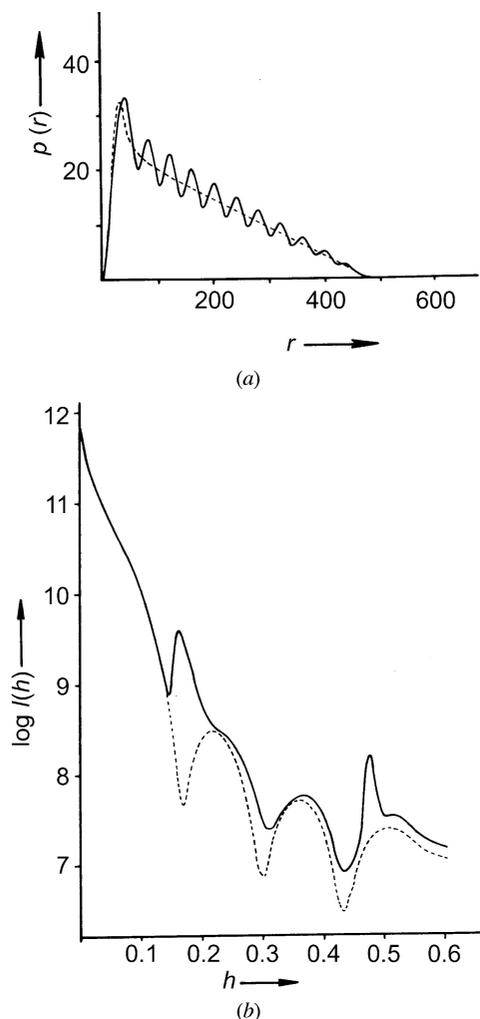


Fig. 2.6.1.9. Inhomogeneous circular cylinder with periodical changes of the electron density along the cylinder axis compared with a homogeneous cylinder with the same mean electron density. (a) $p(r)$ function; (b) scattering intensity; — inhomogeneous cylinder; - - - homogeneous cylinder.

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

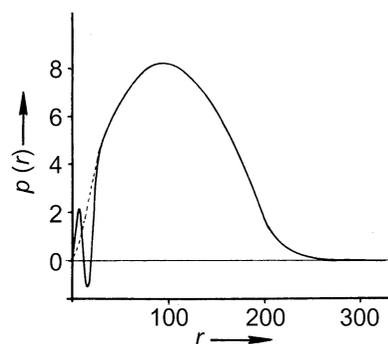


Fig. 2.6.1.10. $p(r)$ function of a lamellar particle. The full line corresponds to an inhomogeneous particle, $\rho_r(x)$ is a three-step function with the values $+1, -1, +1$. The broken line represents the homogeneous lamella with $\rho = +\frac{1}{3}$.

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describe the situation by introducing a radial interparticle distribution function $P(r)$ (Zernicke & Prins, 1927; Debye & Menke, 1930). Each particle has the same surroundings. We consider one central particle and ask for the probability that another particle will be found in the volume element dV at a distance r apart. The mean value is $(N/v) dV$; any deviation from this may be accounted for by a factor $P(r)$. In the range of impenetrability ($r < D$), we have $P(r) = 0$ and in the long range ($r \gg D$) $P(r) = 1$. So the corresponding equation takes the form

$$I(h) = NI_1(h) \left[1 + \frac{N}{V} \int_0^\infty 4\pi r^2 [P(r) - 1] \frac{\sin(hr)}{hr} dr \right]. \quad (2.6.1.53)$$

The second term contains all interparticle interferences. Its predominant part is the 'hole' of radius D , where $[P(r) - 1] = -1$. This leads to a decrease of the scattering intensity mainly in the central part, which results in a liquid-type pattern (Fig. 2.6.1.11). This can be explained by the reduction of the contrast caused by the high number of surrounding particles. Even if a size distribution for the spheres is assumed, the effect remains essentially the same (Porod, 1952). Up to now, no exact analytical expressions for $P(r)$ exist. The situation is even more complicated if one takes into account attractive or repulsive interactions or non-spherical particle shapes (orientation).

If we have a system of spheres with known size D , we can use equation (2.6.1.37) for $I_1(h)$ in equation (2.6.1.53), divide by this function, and calculate $P(r)$ from experimental data by Fourier inversion. The interference term can be used to study particle correlations of charged macromolecular solutions (Chen, Sheu, Kalus & Hoffmann, 1988).

If there are attractive forces, there will be a tendency for aggregation. This tendency may, for instance, be introduced by some steps in the procedure of preparation of biological samples. Such aggregation leads to an increase of the intensity in the central part (gas type). In this case, we will finally have a polydisperse system of monomers and oligomers. Again, there exist no methods to analyse such a system uniquely.

Elimination of concentration effects - liquid type. In most cases, the interference effect is a perturbation of our experiment where we are only interested in the particle scattering function. Any remaining concentration effect would lead to errors in the resulting parameters. As we have seen above, the effect is essential at low h values, thus influencing $I(0)$, R_g , and the PDDF at large r values.

The problem can be handled for the liquid-like type in the following way. We measure the scattering function $I(h)$ at different concentrations (typically from a few mg ml^{-1} up to about 50 mg ml^{-1}). The influence of the concentration can be seen in a common plot of these scattering curves, divided by

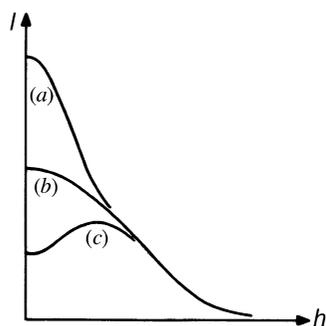


Fig. 2.6.1.11. Characteristic types of scattering functions: (a) gas type; (b) particle scattering; (c) liquid type.

their concentrations. For large h values, these curves are identical. In the low- h range, the curves must be extrapolated to zero concentration. It depends on the problem as to whether a linear fit is sufficient or whether a second-degree polynomial has to be used. The extrapolation can be performed in a standard $I(h)/c$ versus h plot or in a Zimm plot $[I(h)/c]^{-1}$ versus h (Cleemann & Kratky, 1960; Kirste & Oberthür, 1982). The Zimm plot should be preferred when working with highly concentrated solutions (Pilz, 1982).

As mentioned above, the innermost part of the scattering function is lowered and the apparent radius of gyration decreases with increasing concentration. The length of the linear range of the Guinier plot can be extended by the interference effect for non-spherical particles. Thus, an elongated linear Guinier plot is no guarantee of the completeness of the elimination of the concentration effect. Remaining interparticle interferences cannot be recognized in reciprocal space.

The PDDF is affected considerably by interparticle interference (Glatter, 1979). It is lowered with increasing distance r , goes through a negative minimum in the region of the maximum dimension D of the particle, and the oscillations vanish at larger r values. This is shown for the hard-sphere model in Fig. 2.6.1.12. The oscillations disappear when the concentration goes to zero.

The same behaviour can be found from experimental data even in the case of non-spherical data (Pilz, Goral, Hoylaerts, Witters & Lontie, 1980; Pilz, 1982).

In some cases, it may be impossible to carry out experiments with varying concentrations. This will be the case if the structure of the particles depends on concentration. Under certain circumstances, it is possible to find the particle parameters by neglecting the innermost part of the scattering function influenced by the concentration effect (Müller & Glatter, 1982).

Aggregates - gas type. When the particles show a tendency to aggregation with increasing concentration, we can follow the same procedures as discussed for the liquid type, *i.e.* perform a concentration series and extrapolate the $I(h)/c$ curves to zero concentration.

However, in most cases, the tendency to aggregation exists at any concentration, *i.e.* even at very high dilution we have a certain number of oligomers coexisting with monomers. There is no unique way to find the real particle parameters in these cases. It is not sufficient just to neglect the innermost part of the

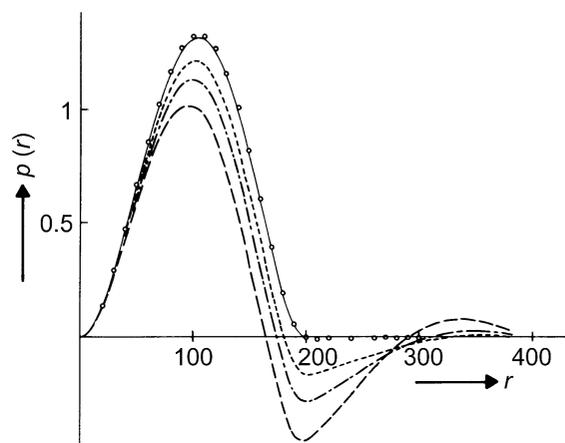


Fig. 2.6.1.12. Distance distribution - hard-sphere interference model. Theoretical $p(r)$ functions: — $\theta = 0$; - - - $\theta = 0.25$; - · - $\theta = 0.5$; — — — $\theta = 1.0$. Circles: results from indirect transformation: $\theta = 0.5$, $h_1 R = 2.0$. 2% statistical noise, $D_{\max} = 300 \text{ \AA}$, $\Delta R_g = 0.5\%$, $\Delta I_0 = 1.2\%$.

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scattering function because that leads to an increasing loss of essential information about the particle (monomer) itself.

2.6.1.4. Polydisperse systems

In this subsection, we give a short survey of the problem of polydispersity. It is most important that there is no way to decide from small-angle scattering data whether the sample is mono- or polydisperse. Every data set can be evaluated in terms of monodisperse or polydisperse structures. Independent *a priori* information is necessary to make this decision. It has been shown analytically that a certain size distribution of spheres gives the same scattering function as a monodisperse ellipsoid with axes a , b and c (Mittelbach & Porod, 1962).

The scattering function of a polydisperse system is determined by the shape of the particles and by the size distribution. As mentioned above, we can assume a certain size distribution and can determine the shape, or, more frequently, we assume the shape and determine the size distribution. In order to do this we have to assume that the scattered intensity results from an ensemble of particles of the same shape whose size distribution can be described by $D_n(R)$, where R is a size parameter and $D_n(R)$ denotes the number of particles of size R . Let us further assume that there are no interparticle interferences or multiple scattering effects. Then the scattering function $I(h)$ is given by

$$I(h) = c_n \int_0^{\infty} D_n(R) R^6 i_0(hR) dR, \quad (2.6.1.54)$$

where c_n is a constant, the factor R^6 takes into account the fact that the particle volume is proportional to R^3 , and $i_0(hR)$ is the normalized form factor of a particle size R . In many cases, one is interested in the mass distribution $D_m(R)$ [sometimes called volume distribution $D_c(R)$]. In this case, we have

$$I(h) = c_m \int_0^{\infty} D_m(R) R^3 i_0(hR) dR. \quad (2.6.1.55)$$

The solution of these integral equations, *i.e.* the computation of $D_n(R)$ or $D_m(R)$ from $I(h)$, needs rather sophisticated numerical or analytical methods and will be discussed later.

The problems of interparticle interference and multiple scattering in the case of polydisperse systems cannot be described analytically and have not been investigated in detail up to now. In general, interference effects start to influence data from small-angle scattering experiments much earlier, *i.e.* at lower concentration, than multiple scattering. Multiple scattering becomes more important with increasing size and contrast and is therefore dominant in light-scattering experiments in higher concentrations.

A concentration series and extrapolation to zero concentration as in monodisperse systems should be performed to eliminate these effects.

2.6.1.5. Instrumentation

X-ray sources are the same for small-angle scattering as for crystallographic experiments. One can use conventional generators with sealed tubes or rotating anodes for higher power. For the vast majority of applications, an X-ray tube with copper anode is used; the wavelength of its characteristic radiation (Cu $K\alpha$ line) is 0.154 nm. Different anode materials emit X-rays of different characteristic wavelengths.

X-rays from synchrotrons or storage rings have a continuous wavelength distribution and the actual wavelength for the experiment is selected by a monochromator. The intensity is much higher than for any type of conventional source but

synchrotron radiation is available only at a few places in the world. Reviews on synchrotron radiation and its application have been published during recent years (Stuhrmann, 1978; Holmes, 1982; Koch, 1988). In these reviews, one can also find some remarks on the general principles of the systems including cameras and special detectors.

2.6.1.5.1. Small-angle cameras

General. In any small-angle scattering experiment, it is necessary to illuminate the sample with a well defined flux of X-rays. The ideal condition would be a parallel monochromatic beam of negligible dimension and very high intensity. These theoretical conditions can never be reached in practice (Pessen, Kumosinski & Timasheff, 1973). One of the main reasons is the fact that there are no lenses as in the visible range of electromagnetic radiation. The refractive index of all materials is equal to or very close to unity for X-rays. On the other hand, this fact has some important advantages. It is, for example, possible to use circular capillaries as sample holders without deflecting the beam. There are different ways of constructing a small-angle scattering system. Slit, pinhole, and block systems define a certain area where the X-rays can pass. Any slit or edge will give rise to secondary scattering (parasitic scattering). The special construction of the instrument has to provide at least a subspace in the detector plane (plane of registration) that is free from this parasitic scattering. The crucial point is of course to provide the conditions to measure at very small scattering angles.

The other possibility of building a small-angle scattering system is to use monochromator crystals and/or bent mirrors to select a narrow wavelength band from the radiation (important for synchrotron radiation) and to focus the X-ray beam to a narrow spot. These systems require slits in addition to eliminate stray radiation.

Block collimation - Kratky camera. The Kratky (1982a) collimation system consists of an entrance slit (edge) and two blocks - the *U*-shaped centre piece and a block called *bridge*. With this system, the problem of parasitic scattering can be largely removed for the upper half of the plane of registration and the smallest accessible scattering angle is defined by the size of the entrance slit (see Fig. 2.6.1.13). This system can be integrated in an evacuated housing (Kratky compact camera) and fixed on the top of the X-ray tube. It is widely used in many laboratories for different applications. In the Kratky system, the X-ray beam has a rectangular shape, the length being much larger than the width. Instrumental broadening can be corrected by special numerical routines. The advantage is a relatively high primary-beam intensity. The main disadvantage is that it cannot be used in special applications such as oriented systems where

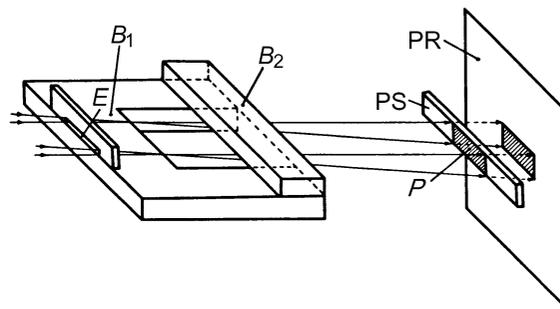


Fig. 2.6.1.13. Schematic drawing of the block collimation (Kratky camera): E edge; B_1 centre piece; B_2 bridge; P primary-beam profile; PS primary-beam stop; PR plane of registration.