INTERNATIONAL STANDARD

ISO 17867

First edition
2015-05-01

Particle size analysis — Small-angle X-ray scattering

Analyse granulométrique — Diffusion des rayons X aux petits angles

Reference number
ISO 17867:2015(E)

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT), see the following URL: Foreword — Supplementary information.

The committee responsible for this document is ISO/TC 24, *Particle characterization including sieving*, Subcommittee SC 4, *Particle characterization*.

Introduction

This International Standard deals with Small-Angle X-ray Scattering (SAXS), which is performed for particle size analysis in the 1 nm to 100 nm size range. In ideal circumstances, it can provide an estimate of particle size, average size and its distribution, surface area, and sometimes particle shape in a reasonably rapid measurement time. User-friendly commercial instruments are available worldwide from a number of manufacturers for both routine and more sophisticated analyses, and state-of-the-art research instruments are available at synchrotron radiation facilities.

As in all particle size measurement techniques, care is required in all aspects of the use of the instrument, collection of data, and further interpretation. Therefore, there is a need for an International Standard that allows users to obtain good inter-laboratory agreement on the accuracy and reproducibility of the technique.

SAXS can be applied to any hetero-phase system, in which the two or more phases have a different electron density. In most cases, the electron density corresponds reasonably well to the mass density. The so-called ‘particle’ is always the phase with the smaller volume fraction. Because SAXS is sensitive to the squared electron density difference, it does not matter whether the particles constitute the denser phase and the solvent (or matrix) is the less-dense phase or vice versa. Thus, pore size distributions can be measured with SAXS in the same way as size distributions of oil droplets in emulsions or solid particles in suspensions.

Although SAXS allows the determination of particle size, size distribution, surface area, and sometimes particle shape in concentrated solutions, in powders and in bulk materials, this International Standard is limited to the description of particle sizes in dilute systems. A dilute system in the sense of SAXS means that particle interactions are absent. In case of long range interactions (Coulomb forces between the particles), special care has to be taken and a reduction of the concentration or the addition of salt might be necessary.

Since all illuminated particles present in the X-ray beam are measured simultaneously, SAXS results are ensemble and time averaged across all the particle orientations which are present in the sample.

The shape of the particles can be assigned to a basic geometry: spheroid, disk, or cylinder. This does not exclude more detailed information about the shape of the particle being obtained. However, the method of calculation for more detailed shape analysis is very complex to be included in an International Standard at this time. The sizes of irregularly shaped nanoparticles can be assessed by the radius of gyration (R_g) as obtained by classic Guinier analysis.

The size and size distribution of particles with basic shapes (sphere, disk, cylinder, core-shell, etc.) can be determined from curve fitting for relatively narrow size distributions. The reliability of the method of calculation for broader distributions depends on prior knowledge of the distribution.

This International Standard assumes isotropically oriented nanoparticles of any shape in a test procedure. No dimension of the nanoparticle shall be larger than defined by the scattering accessible to the specific SAXS instrument. This generally limits the largest measureable particle size of the conventional technique to 100 nm, although this limit can be significantly extended in samples with a very narrow size distribution.

Small-angle neutron scattering is not described in this International Standard, but can be used without restriction because the theory and application are similar.

A list of suitable references for further reading is given in the Bibliography.

Particle size analysis — Small-angle X-ray scattering

1 Scope

Small-angle X-ray scattering (SAXS) is a well-established technique that allows structural information to be obtained about inhomogeneities in materials with a characteristic length from 1 nm to 100 nm. Under certain conditions (narrow size distributions, appropriate instrumental configuration, and idealised shape) the limit of 100 nm can be significantly extended. This International Standard specifies a method for the application of SAXS to the estimation of mean particle sizes in dilute dispersions where the interaction between the particles is negligible. This International Standard allows two complementary data evaluation methods to be performed, model fitting and Guinier approximation. The most appropriate evaluation method shall be selected by the analyst and stated clearly in the report. SAXS is sensitive to electron density fluctuations. Therefore, particles in solution and pores in a matrix can be studied in same way.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 26824, *Particle characterization of particulate systems — Vocabulary*

ISO 9276-1, *Representation of results of particle size analysis — Part 1: Graphical representation*

ISO 9276-2, *Representation of results of particle size analysis — Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions*

ISO/TS 27687, *Nanotechnologies — Terminology and definitions for nano-objects — Nanoparticle, nanofibre and nanoplate*

3 Symbols and abbreviations

Table 1 — Symbols

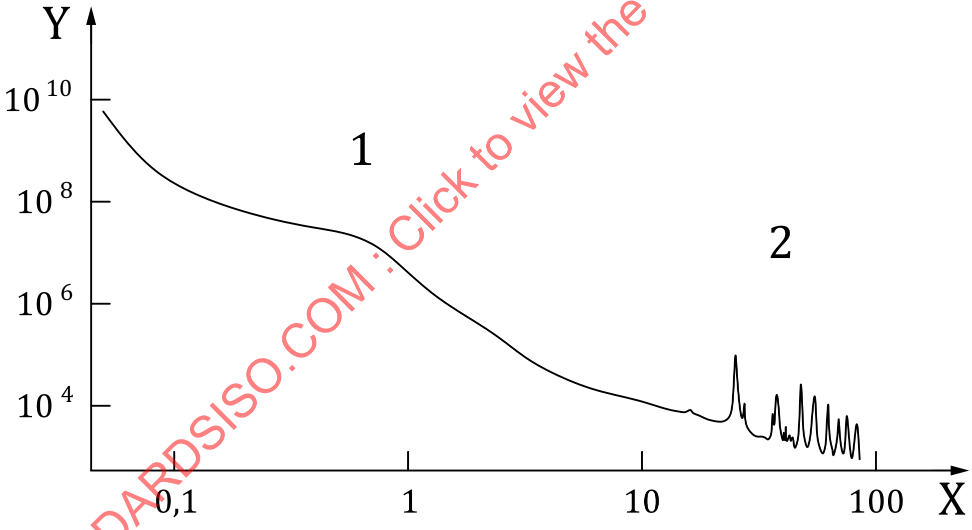
Symbol	Name	Unit
$\overline{d}_{\text{vs}}$	Volume-squared-weighted mean particle diameter	nm
$\overline{d}_{\text{num}}$	Number-weighted mean particle diameter	nm
I_{out}	Primary beam intensity with sample	
I_{in}	Primary beam intensity without sample	
$I(q)$	Scattered intensity (or scattering intensity)	
q	Momentum transfer or q-value, magnitude of the scattering vector given by $q = (4\pi /\lambda)\sin\theta$	nm^{-1}
r	Particle radius	nm
R_g	Radius of gyration (Guinier radius, see A.4)	nm
t_o	Optimum sample thickness	mm
T	Transmission	

Table 1 *(continued)*

Symbol	Name	Unit
V	Volume of particle	nm^3
λ	Wavelength of the incident X-rays in vacuum	nm
2θ	Scattering angle	deg or rad
μ	Linear absorption coefficient	mm^{-1}
σ	Standard deviation of size distribution	

4 Principle of the method

When electromagnetic radiation impinges on matter, a small fraction of the radiation is scattered. As a function of the scattering angle or momentum transfer, q-value, the scattered radiation intensity profile contains information that can be used to obtain various characteristics of the material. In particular, when X-rays impinge on a geometrically ordered group of particles or molecules, this gives rise to the well-known X-ray diffraction pattern at wide scattering angles which is used to characterize the unit cell and lattice constants of the material. In the small-angle regime (typically $2\theta < 5°$; wavelength dependent), information on the size of particles or pores within the material is available from the elastic (no change in wavelength) scattering arising from the electron density contrast between the particles and the medium in which they reside. This is analogous to static light scattering. A diagrammatic form of the angular dependence of the X-ray scattered intensity of a titanium dioxide mixture (rutile and anatase) is shown in Figure 1.



Key

1 SAXS range
2 XRD range
X scattering angle 2θ/deg
Y intensity

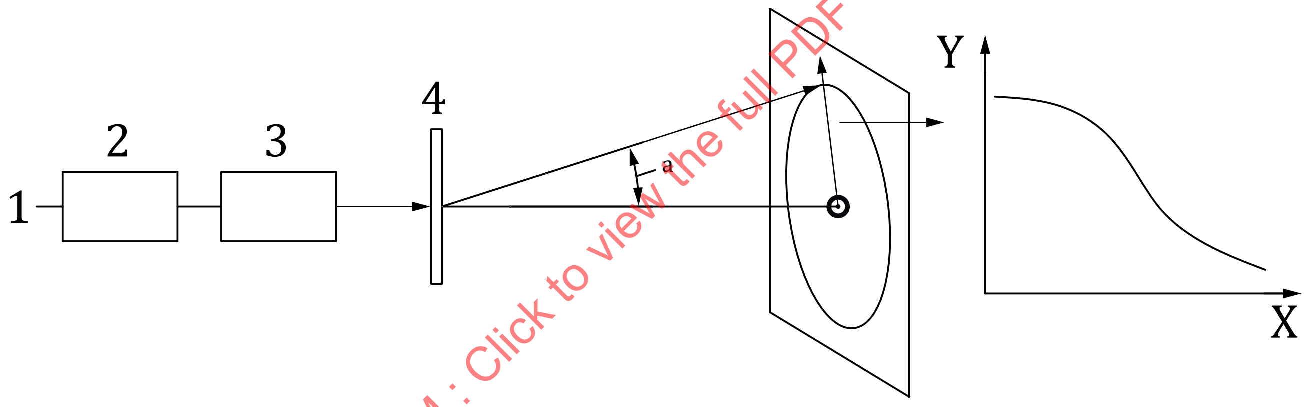
Figure 1 — X-ray scattering diagram illustrating the small-angle SAXS region (left hand side) and the wide-angle XRD region (right hand side) of a titanium dioxide powder

At low concentration, the small-angle scattering region contains information about particle size, size distribution, and particle shape, for which different ranges of q are evaluated. The Guinier approximation can be applied in the low-q range to get an intensity weighted mean size, when the particles are smaller than $2\pi/q_{\text{min}}$. Model fitting can be applied in the full range of q to compute traceable particle size and size distribution with associated uncertainties. Porod's law can be applied to the high-q range to get an indication of the particle shape. This last method does not provide particle size and is therefore outside the scope of this International Standard. Note that all three methods can fail depending on data quality and particle properties.

At increased concentrations, i.e. those higher than typically one volume %, particle-particle interactions and inter-particle interference can be relevant. Such interactions require sophisticated data modelling and expert knowledge for data interpretation, which is beyond the scope of the present standard. In practice, a concentration ladder may be explored in order to determine the dependence of reported size on concentration. If available, each sample shall be measured twice: in its original concentration, and diluted 1:1 to exclude concentration artefacts. The result of both measurements shall be arithmetically averaged and the uncertainty enhanced by the variation. If dilution is not possible for technical reasons, this shall be stated in the report and the uncertainty shall be marked. Note that the radius of gyration is more affected by concentration than model fitting.

5 Apparatus and procedure

A diagrammatic form of a SAXS instrument is shown in Figure 2.



Key

1	X-ray source
2	optics
3	collimation system
4	sample
a	2θ
X	2θ or q
Y	scattered intensity

Figure 2 — Diagrammatic form of a SAXS instrument, consisting of X-ray source, optics, collimation system, sample holder, beam stop, and X-ray detector

The SAXS set-up consists of X-ray source, optics, collimation system, sample holder, beam stop, and detector. In order to extract meaningful information from the measurement, the following key parameters define the capability of the system:

- q-range: q_{min} and q_{max}; number of sampled points in the Guinier region for Guinier approximation;
- detector sensitivity and system background noise.

Most available X-ray sources produce divergent beams which shall be collimated for SAXS measurements. With laboratory X-ray sources, multilayer optics are commonly used but basic SAXS measurements can also be achieved with slit collimation. The X-ray flux on the sample is generally higher when optics is used. Furthermore, multilayer coated optics can be used to generate a monochromatic X-ray beam.

The greatest challenge in SAXS is to separate the incoming primary beam from the scattered radiation at small angles (around 0.1°). The direct beam should be blocked by a beam stop and parasitic scattering should be eliminated. The need for separation of primary and scattered beam makes collimation of the primary beam mandatory.

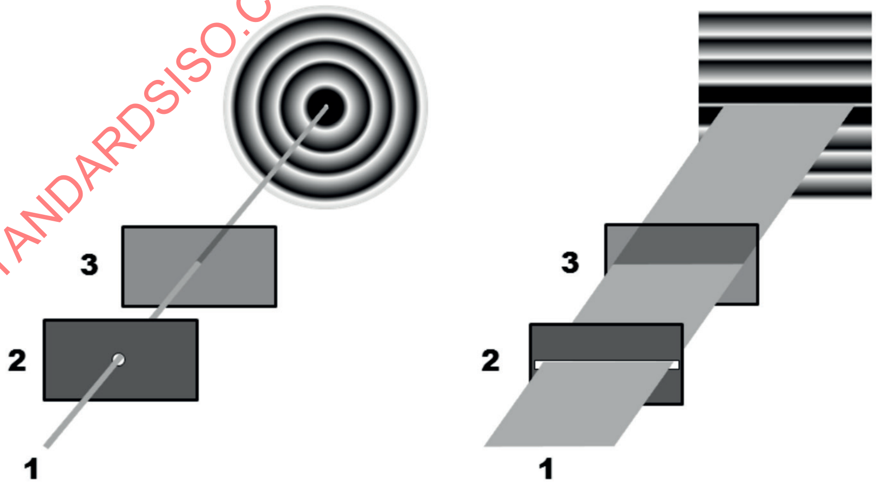
There are two main options to collimate an X-ray beam (see Figure 3):

— Point collimation systems have pinholes or crossed slits that shape the X-ray beam to a small dimension (typically, the beam spot on the sample is less than 0,8 mm in diameter) that illuminates the sample. The scattering is centro-symmetrically distributed around the primary X-ray beam. For isotropic samples, the scattering pattern in the detection plane consists of circles around the primary beam. The illuminated sample volume is smaller than in line-collimation. Point collimation allows the study of isotropic and anisotropic systems.

— Line-collimation instruments confine the beam in one dimension so that the beam profile is a long and narrow line. The beam dimension can be adjusted according to the type of sample for studies. Typical dimensions are 20 mm × 0,3 mm. The illuminated sample volume is larger compared to point-collimation and the scattered intensity at the same flux density is proportionally larger. If the system is isotropic, the resulting smearing can be removed using deconvolution. The investigation of anisotropic systems is not as straightforward as for point collimation.

In addition, the point and line collimation systems can use either a parallel or focused beam (see Figure 4).

The majority of the generated X-rays will simply transmit through the diluted sample without interacting with the particles. The X-rays scattered by the particles form a scattering pattern that contains the information on the size and structure of the sample. This pattern is detected typically by a 1- dimensional or 2-dimensional flat X-ray detector situated behind the sample and perpendicular to the direction of the primary beam. Some multipurpose diffractometers that combine SAXS and diffraction use a scanning point detector. There are a number of types of detector routinely employed, for instance, photon-counting and integration type detectors. The scattering pattern contains the information on the structure of the sample.



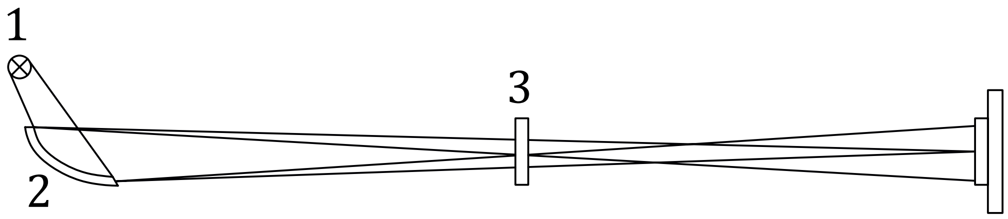
Key

1 X-ray source

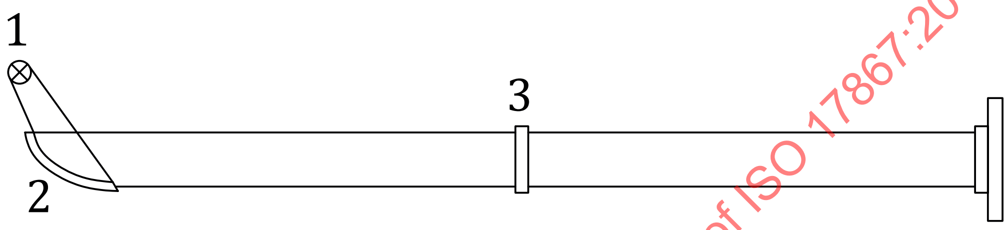
2 collimation system

3 sample

Figure 3 — Point and line collimation types used in SAXS



a) Point (2D) or line (1D) focused beam



b) Parallel beam

Key

1	X-ray source
2	mirror
3	sample

Figure 4 — Focused and parallel beam set-up

6 Preliminary procedures and instrument set-up

Wavelength calibration (see Annex C) can be performed before conducting an experiment and thus would be classified as a preliminary procedure, but this is not routinely done in the laboratory. If characteristic X-rays of copper are used, a nickel absorber can be used to check that Cu Kα radiation has been selected correctly. Utilization of calibration materials, for example, silver behenate, should form part of a full system qualification and fit-for-purpose specification as noted in Annex C.

7 Sample preparation

Sample preparation is simple and fast for SAXS measurements. The required sample volumes are small, typically in a range of 5 μL to 50 μL for liquids and pastes, if copper radiation is used. Solid samples require an area of (1 × 1) mm^2 to (1 × 20) mm^2. The sample thickness is typically smaller than 1 mm.

Liquid samples are usually measured inside a thin-walled capillary, the diameter of which is around 1 mm to 2 mm when the liquid primarily contains water or hydrocarbons. Solvents that contain heavy atoms, for example, chlorine in chloroform, should be measured in smaller diameter capillaries as the atoms strongly absorb the incident radiation. Viscous samples can be measured better in a paste cell.

Pastes, powders, and vacuum sensitive materials can be mounted into a sample holder with windows, which shall be transparent to X-rays and exhibit little scattering themselves. Frequently used window materials include polyimide foils. Care should be taken that the scattering from the window material does not affect the result of the measurement. Polyimide films exhibit a broad small-angle diffraction peak in the vicinity of q approximately 0,7 nm^{-1}, which has to be taken into account in data interpretation.

Solids can be clamped onto frames with or without additional window foils for protection against the vacuum. The sample thickness shall be chosen in line with the respective absorption of the material.[14] The optimum thickness, t_0, is given by

$$t_o = 1 / \mu \tag{1}$$

where μ is the linear absorption coefficient of the material. The optimum specimen thickness corresponds to a ratio of the primary beam intensity with and without sample, I_{out} and I_{in}, of:

$$I_{out} / I_{in} = e^{-\mu t} = e^{-1} \sim 37\% \tag{2}$$

Thus, the ideal specimen will transmit about 37 % of the incident radiation, and the specimen thickness can be adjusted accordingly to optimize transmission. Any sample treatment (for example, dilution, sonication, or centrifugation) may affect the particle size distribution and should be described in the analysis report.

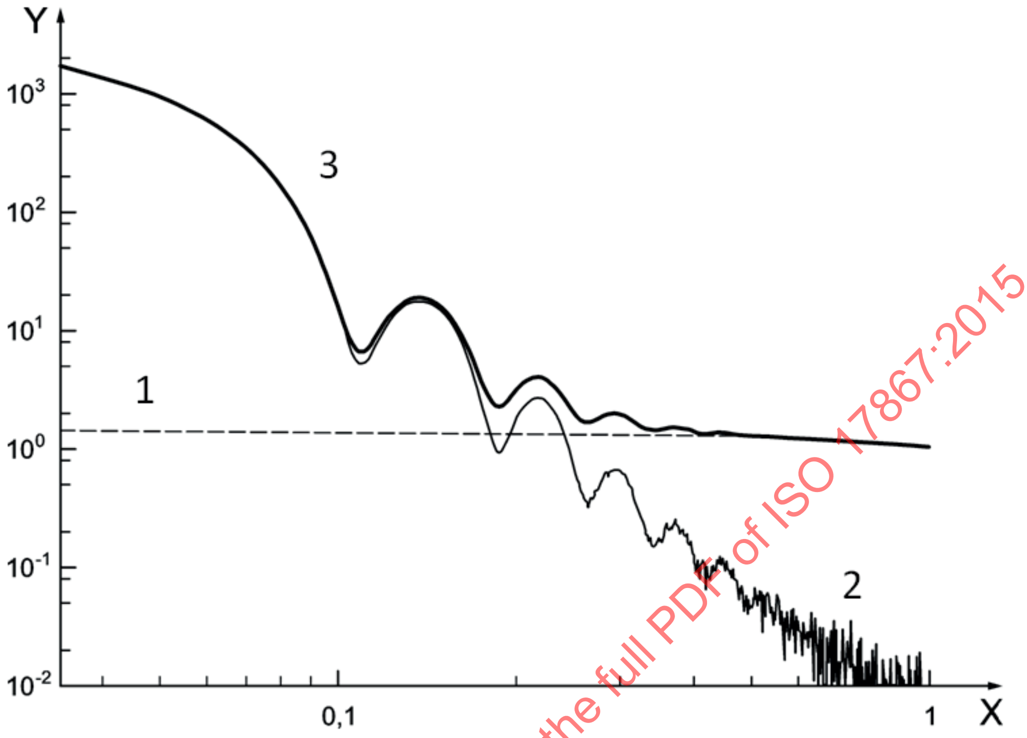
8 Measurement procedure

Every SAXS particle-sizing experiment consists of at least two measurements using the same sample holder and the same acquisition time:

a) sample scattering;

b) solvent/matrix scattering, the so-called blank or background experiment.

This is the minimum requirement for determining the scattering of the particles, which is the difference between the two scattering measurements. A typical example for this procedure is given in Figure 5. Care has to be taken that the scattering of the window material of the sample cell, the parasitic scattering of the SAXS instrument, and the dark count rate of the detector are removed. The transmission from the sample and background/matrix material and efficiency variation over the detector shall be taken into account.



Key

1	solvent
2	particle scattering
3	particle dispersion
X	q/nm^{-1}
Y	scattered intensity $I(q)$

Figure 5 — Typical SAXS profiles of a particle dispersion, the solvent and the difference (the corrected signal only due to particle scattering)

The statistical quality of the scattering pattern improves with increasing intensity and complies with standard statistics for signals obtained by the subtraction of two independent measurements.

9 Calculation of the mean particle diameter

9.1 General

After background subtraction and desmearing (if required, see Annex A), the mean particle diameter can be calculated according to two different approaches.

9.2 Guinier approximation

For the Guinier approximation (explained in detail in A.3), ln(I) is plotted as a function of q^2 (Guinier plot). As the scattered intensity at very small angles is approximated by a Gaussian function

$$I(q) = I_0 \exp\left[-\frac{1}{3}R_g^2 q^2\right] \tag{3}$$

which can be transformed to

$$\ln\left[I(q)\right] = \ln\left[I_0\right] - \frac{1}{3}R_g^2 q^2 \tag{4}$$

A straight line can fit the data in the Guinier region which is typically up to qR_g around 1. The slope is then equal to $-\frac{1}{3}R_g^2$

For monodisperse homogeneous spherical particles, the volume-squared-weighted mean particle diameter can be calculated from R_g according to:

$$\bar{d}_{vs} = 2\sqrt{\frac{5}{3}}R_g \tag{5}$$

According to ISO 9276-2 and Reference [3], $\bar{d}_{vs}$ corresponds to $\bar{D}_{8,6}$ and $\bar{x}_{2,6}$.

9.3 Model fitting

For model fitting, the full range of q can be fitted by a model function for a polydisperse ensemble of particles according to:

$$I(q) = N\int_0^\infty P(q,r)g(r)dr + c \tag{6}$$

where

N is a scaling factor (including the number of particles, the electron density difference, the intensity of the primary beam etc.);

$P(q,r)$ is the form factor;

$g(r)$ represents the distribution and c a constant background.

For homogenous spheres, the form factor is given by:

$$P(q,r)=\left(\frac{4\pi}{q^3}(\sin qr - qr\cos qr)\right)^2 \tag{7}$$

The most common distributions are lognormal and Gaussian: A Gaussian size distribution is described by:

$$g(r)=\exp\left(\frac{\left(r-\bar{d}_{\mathrm{num}}/2\right)^2}{2\sigma^2}\right) / \int_0^\infty \exp\left(\frac{\left(\rho-\bar{d}_{\mathrm{num}}/2\right)^2}{2\sigma^2}\right) d\rho \tag{8}$$

and a lognormal distribution can be written as:

$$g(r)=\frac{1}{\sqrt{2\pi}\, r\sigma_{\ln}}\exp\left(-\frac{\left(\ln r-\ln\left(\frac{1}{2}\bar{d}_{\ln}\right)\right)^2}{2\sigma_{\ln}^2}\right) \tag{9}$$

where the mean diameter $\bar{d}_{\ln}$ can be transformed to the number-weighted mean particle diameter of a Gaussian distribution $\bar{d}_{\mathrm{num}}$ according to:

$$\bar{d}_{\mathrm{num}}=\bar{d}_{\ln}\exp\left(\sigma_{\ln}^2/2\right) \tag{10}$$

N, c, the standard deviation of the size distribution (σ or $\sigma_{\ln}$) and the mean particle diameter ($\bar{d}_{\mathrm{num}}$ or $\bar{d}_{\ln}$) are the fit parameters. From the determined size distribution, also the volume-weighted and intensity-weighted mean particle diameters can be calculated.

According to ISO 9276-2, $\bar{d}_{\mathrm{num}}$ corresponds to $\bar{D}_{1,0}$ and $\bar{x}_{1,0}$.

Information on the particle size can also be obtained from other evaluation methods in real space or Fourier space as explained in A.5.

10 Repeatability

Repeated measurements of the same sample can indicate if the material is changing during the duration of the experiment and therefore can be an indicator of degradation under the X-ray beam. Additionally, sample-to-sample measurements will indicate homogeneity or heterogeneity of the material. Sample-to-sample heterogeneity and instability of a sample/material over time can only be detected if the heterogeneity and instability create effects that can be distinguished beyond the method repeatability. The method repeatability shall be established for each individual instrument in a validation study on a suitable, calibration standard. A frequently used material is silver behenate.

11 Documentation and test report

11.1 Test report

Test reports should be prepared in line with ISO 9276-1 and ISO 9276-2. The sections "identification of the method used", "test results", and "description of the test item" in the scope of this International Standard contain the following information:

a) reference to this International Standard;

b) the mean particle diameter $\bar{d}$ and its uncertainty, including a clear statement whether this represents a number, volume, or intensity weighted mean. In the absence of a full uncertainty evaluation, the standard deviation from several repeated measurements should be provided as estimate of the repeatability. ISO/IEC Guide 98-3 can assist here, but expert judgment may have to be employed.

The standard error of the slope, u_{slope}, can be obtained as a linear regression analysis output from many statistical software packages and some graphing calculators. The uncertainty of R_g is calculated as:

$$u_{\text{Rg}} = -3\left(u_{\text{slope}}\right)/\left(2R_g\right) \tag{11}$$

It is noted that the slope itself, its uncertainty u_{slope} and the corresponding u_{Rg} can vary with the part of the Guinier region that is selected for analysis.

c) if a particle size distribution is included, the assumptions in deriving this distribution should be explained in a short description. A graphical plot should be in accordance with ISO 9276-2:2014;

d) complete sample identification, including available information on particle shape and homogeneity. Electron micrographs, where relevant and informative, can be included in order to convey information on particle shape, degree of dispersion, crystallinity, and other visual indicators that are not easily conveyed in graphical or tabular data;

e) applied data evaluation (Guinier approximation for model fitting);

f) form factor and size distribution if model fitting is used;

g) results including mean diameter and uncertainty for two samples of differing concentration (if available);

h) range of q selected for evaluation.

11.2 Technical records

In addition to the information given in the test report, the following information on the measurements should be documented in line with the provisions on technical records as stated in ISO/IEC 17025. These records shall be readily retrievable and should be provided to the customer on request:

a) instrument type and serial number;

b) dispersion and dilution procedures, including nature, concentration and quantities of liquids and their cleaning procedure, if applicable;

c) concentration of particulate material in the dispersions, if applicable;

d) measurement conditions;

e) temperature of the sample;

f) analyst identification (name or initials).

Annex A
(informative)

General principles

NOTE The calculation of the particle size is a sequential process as indicated earlier.

A.1 Background correction

The particle scattering intensity, $I_{\mathrm{particle}}(q)$, required for the data interpretation is obtained by subtracting the blank scattering I_{blank}, from the sample scattering, I_{sample}:

$$I_{\mathrm{particle}}(q) = I_{\mathrm{sample}}(q) - I_{\mathrm{blank}}(q) \tag{A.1}$$

The blank scattered contains matrix scattering, $I_{\mathrm{M}}(q)$, incoherent scattering, I_{IC}, and the detector dark count, I_{DC}. This simple procedure can be applied only for diluted particle systems. If the transmission of sample and blank are different, this has to be taken into account.[10][24]

Special care shall be taken to prevent parasitic instrumental scattering, which can become predominant in the forward direction close to the primary beam stop. Any data points for which the blank curve contributes more than 50 % of the total scattering should be used with caution due to the loss of accuracy in the background-subtraction process.

A.2 Data interpretation

The observed scattered intensity after background subtraction is related to the particle scattering by

$$I_{\mathrm{particle}}(q) = N\left|F(q)\right|^2 \tag{A.2}$$

where the scattering amplitude $F(q) = \int_V \Delta\rho e^{\mathrm{iqr}} dV$ depends on the particle shape. The $\Delta\rho$ is the difference between the electron densities of the scatter and the matrix, and V is the volume of the particle.[11] The form factor $P(q) = \left|F(q)\right|^2$ contains information on electron density distribution and shape of the particle.

The radius of gyration is determined by straight-line fitting and represents a mass-fraction weighted mean value of the underlying size distribution. It can be easily applied only if a sufficiently large linear region can be found in the scattering data. This is the case when the width of the size distribution is below 20 %.

The maximum ($D_{\max}$) and minimum dimensions ($D_{\min}$) of the nanoparticle shall fit into the experimental window of scattering angles specific to the used SAXS instrument. As a good first-order approximation, one can use:

$$q_{\max} R_{\mathrm{g}} >\approx 5$$

or that

$$q_{\max} >\approx 4\pi / D_{\min}$$

This International Standard recommends for the Guinier approximation measurements in the range of the limiting scattering angles between $q_{\min}$ and $q_{\max}$. All particles or aggregates with dimensions larger than those specified by these two limits will either not be recorded at all (if $D_{\max} > \frac{2\pi}{q_{\min}}$), because their size information is not in the signal, or will disturb the Guinier analysis by adding non-Gaussian contributions to the signal, which cannot be fitted with Guinier's method.

For point-collimation instruments, the desmearing of data is not required. Therefore, the experimental data can be directly used for data modelling or Guinier's analysis. When using line-collimated instruments, special care shall be taken for the presence of instrumental broadening due to collimation effects. Notable influences on the data are beam-dimensions (beam length and beam width), detector cross-talk (point-spread functions) and wavelength distributions. Because of practical constraints, such as limited beam time and through-put, it is not always possible to circumvent non-ideal instrumental conditions of beam dimension. It is therefore recommended to use only evaluation software, which is capable of dealing with the specific instrumental conditions. There are two ways of implementing these requirements, which includes.

a) desmearing of experimental data, and

b) smearing of theoretical fitting functions.

Both methods are generally possible. In this International Standard, we recommend the second method due to the application of the specific model of Guinier's approximation.

Small-angle neutron scattering is not included in this International Standard but can be used without restrictions

a) if the distinction between *two phases* is made on the basis of the scattering-length density of the atomic nuclei, rather than the electron density, and

b) if the usual wavelength-distribution width of about $\Delta\lambda/\lambda$, approximately 0,1, is included in the evaluation procedure.

A.3 Calculation of the radius of gyration from Guinier plot

The radius of gyration is defined as the root-mean-square of the distances from all the electrons in the particle to its centre of gravity of the electron distribution.

$$R_g^2 = \frac{\int\limits_v r^2 \rho(r) dV}{\int\limits_v \rho(r) dV} \qquad \text{(A.3)}$$

where $\rho(r)$ is electron density. The pair-distance distribution function (PDDF) $p(r)$ of the particle:

$$p(r) = \left\langle \int\limits_V \Delta\rho(\vec{s}) \Delta\rho(\vec{s} - \vec{r}) \overrightarrow{ds} \right\rangle \qquad \text{(A.4)}$$

and $\Delta\rho(\vec{s}) = \rho_{\text{particle}}(\vec{s}) - \rho_{\text{solvent}}$ is the relative electron density inside the particle at position $\vec{s}$. The integration is taken over the whole particle volume V. The angled brackets indicate averaging over all particle orientations relative to the primary-beam direction.

Particle shapes deviating from spherical can be modelled by spherical harmonics which are amenable to fitting by a range of different algorithms. Obviously, particles of different shapes can possess identical radii of gyration and the value of R_g cannot distinguish between these. For spherical particles:

$$R_g = (3/5)^{1/2} r \qquad \text{(A.5)}$$

where r is the radius of a homogeneous spherical particle.

Thus, radii of gyration shall be multiplied by $(5/3)^{1/2} \sim 1{,}29$ to obtain the radii of the equivalent spheres.

Similarly, it can be shown that the radius-of-gyration equivalence for a homogeneous circular cylinder/disk of radius R and height L is:

$$R_G = \sqrt{R^2/2 + L^2/12} \tag{A.6}$$

The shape of the scattered intensity decay at very small angles is approximated by a Gaussian function

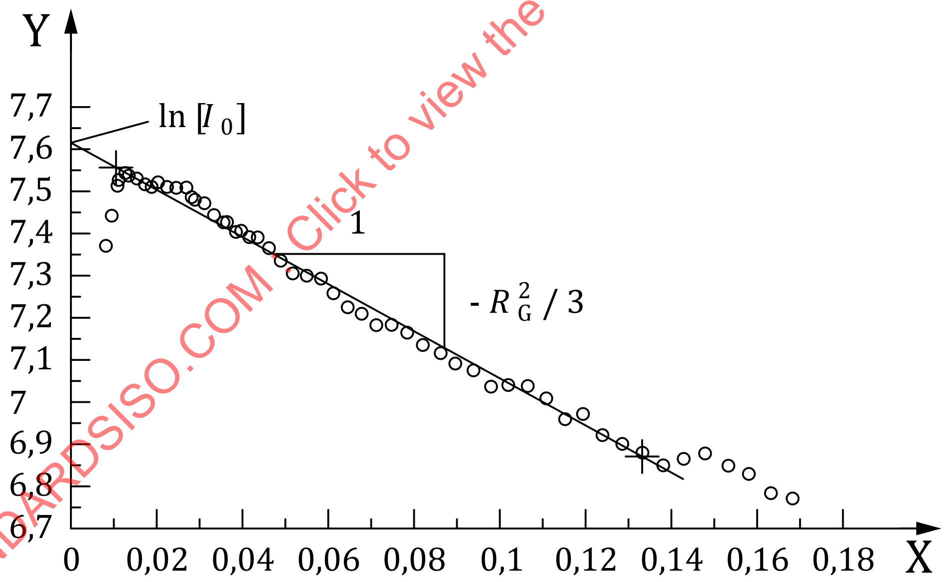
$$I(q) = I_0 \exp\left[-\frac{1}{3}R_g^2 q^2\right] \tag{A.7}$$

where the steepness of the decay is determined by the radius of gyration. This is termed Guinier's law and R_g is termed radius of gyration (Guinier radius).

For further analysis, Formula (A.7) is used in its logarithmic form, leading to the linear equation:

$$\ln\left[I(q)\right] = \ln\left[I_0\right] - \frac{1}{3}R_g^2 q^2 \tag{A.8}$$

in order to determine R_g and the intensity at $q = 0$ from the experimental curves. In this case, ln $[I(q)]$ is plotted as a function of q^2. If a straight line can fit the data, the slope is equal to $-\frac{1}{3}R_g^2$ and the intercept is ln $[I_0]$. Note that the Guinier approximation is valid only for small qR_g.



Key

X q^2/ nm^{-2}

Y ln $[I(q)]$

Figure A.1 — Guinier plot for calculation of particle's radius of gyration R_g and the zero angle intensity I_0

In the above example, the slope is calculated as:

$1/3 \times R_g^2 = (7{,}52 - 6{,}88) / (0{,}132 - 0{,}012)$ nm^2

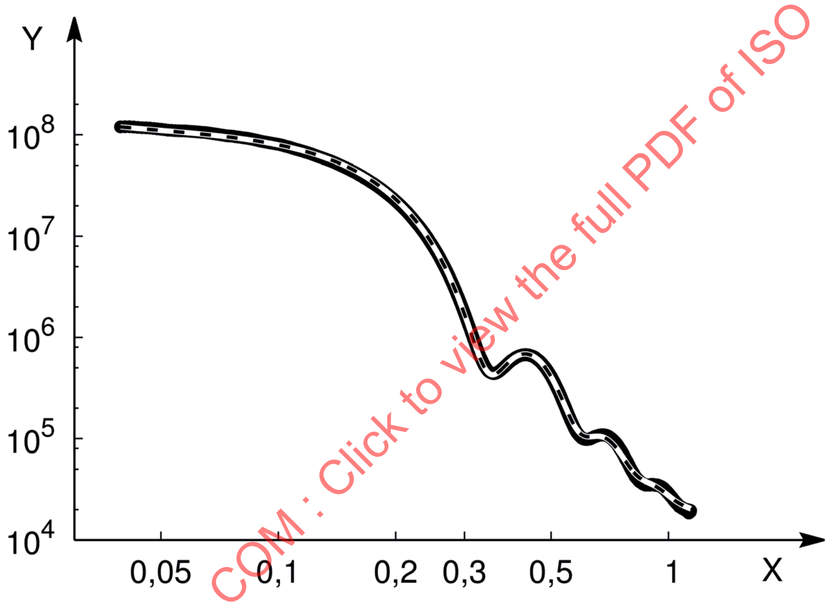
Thus, R_g is 4,0 nm.

If the data points do not lie on a straight line, as will be the case for irregular particles or polydisperse samples, then the Guinier approximation cannot be used, and the calculation of particle size and distribution is a complex issue and beyond the scope of this International Standard.

Note that at very small angles the data points in Figure A.1 suddenly drop below the fitted straight line. This is caused by the beam stop and indicates the low-q limit of the measurement.

A.4 Calculation of the mean diameter from model fitting

The entire q-range can be used to fit the data. The mean particle diameter results from the fit. For homogeneous spherical particle, only the radius determines the period of the oscillations which are observed for sufficiently monodisperse particles. The amplitude of the oscillations is mainly determined by the width of the size distribution. For gold nanoparticles shown as an example in Figure A.2, a number-weighted mean diameter of (25,3 ± 0,5) nm was obtained. For homogeneous spheres, the uncertainty of the diameter is dominated by the fitting procedure.[16]



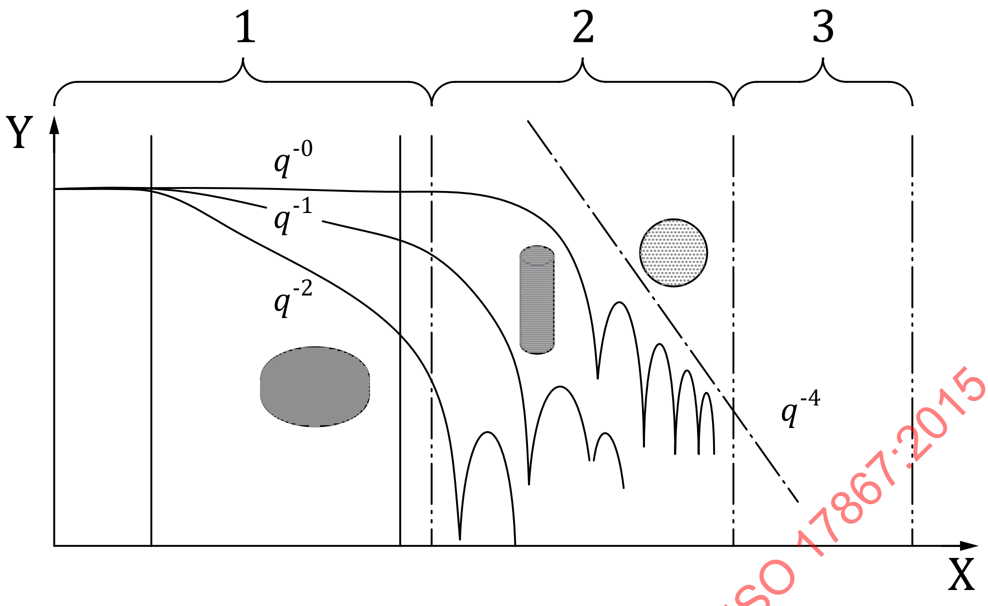
Key

●━━●	measured
– – –	fit
X	q/nm^{-1}
Y	scattered intensity $I(q)$

Figure A.2 — Measured scattered intensity and fit using the form factor for homogeneous spheres with a Gaussian size distribution (from[15])

A.5 Particle shape

Every particle produces a form factor that is characteristic of the structure of the particle. Accurate shape determination from SAXS data is possible for very narrow size distributions. The slope of the form factor at small angles is primarily determined by the overall size and the final slope at large angles is indicative of the surface. Information about the shape and the internal density distribution lies in the oscillating part in the middle section of the form factor:



Key

1 Guinier region
2 Fourier region
3 Porod region
X q/nm^{-1}
Y scattered intensity $I(q)$

Figure A.3 — The information domains of a particle form factor are Guinier, Fourier, and Porod regions

A rough classification into globular, cylindrical, and disk shapes (with axial ratio larger than 5) can be quickly performed by investigating the power law of the form factor at small angles (see Figure A.3). In a double logarithmic plot, an initial slope of 0, −1, or −2 indicates globular, cylindrical, or disk shape respectively. If the slope in this region is steeper (−3 or −4), the particles are too large to be resolved. This means that most of the form factor is too close to the primary beam and the Porod region is the only part of the form factor that is experimentally accessible.

The oscillating part of the form factor can be profitably investigated by curve fitting or transforming the scattering pattern into real space by Fourier transform methods. The resulting curves are called pair-distance distribution functions (PDDF's). A PDDF is a histogram of distances that can be found inside a particle.

See: Reference [8] and Reference [10] (Chapters 2, 4, and 5).

By identifying key features in the PDDF, the shape of a particle can be quickly classified into

i) spherical or globular,

ii) prolate or cylindrical, and

iii) oblate or lamellar symmetry.

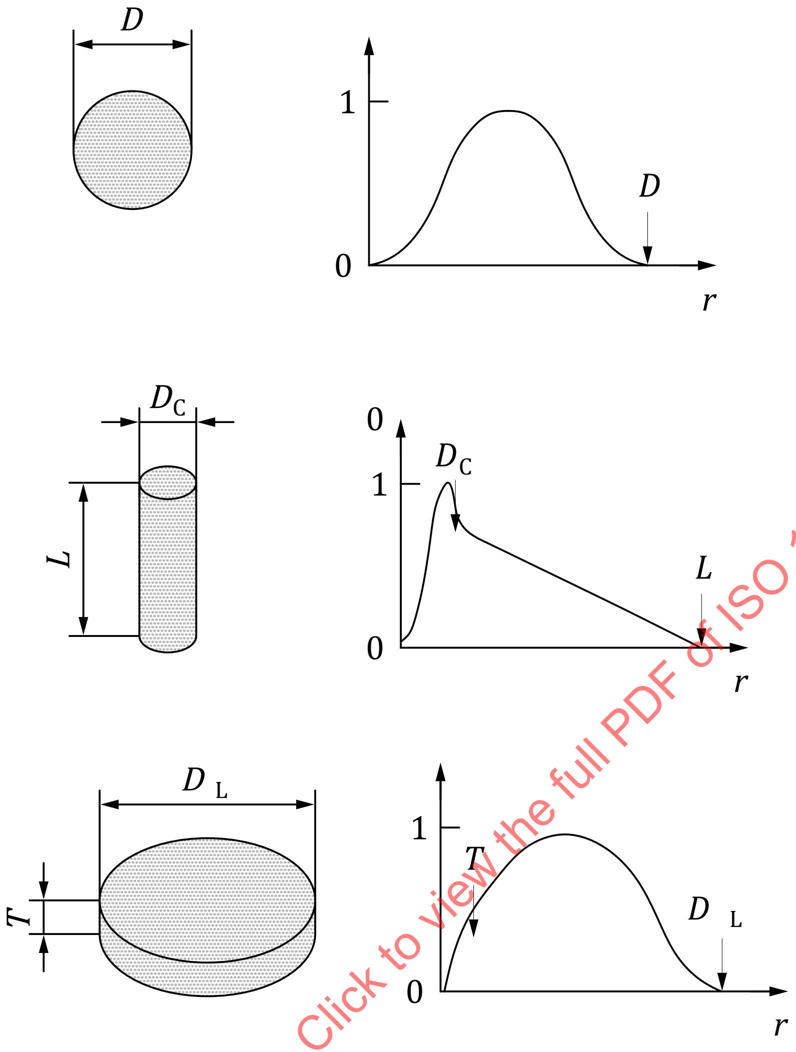


Figure A.4 — Key features of the PDDF indicating spherical, cylindrical, or lamellar particle shape

It is comparatively easy to recognize the PDDF’s of two subunits as these will show a secondary peak:

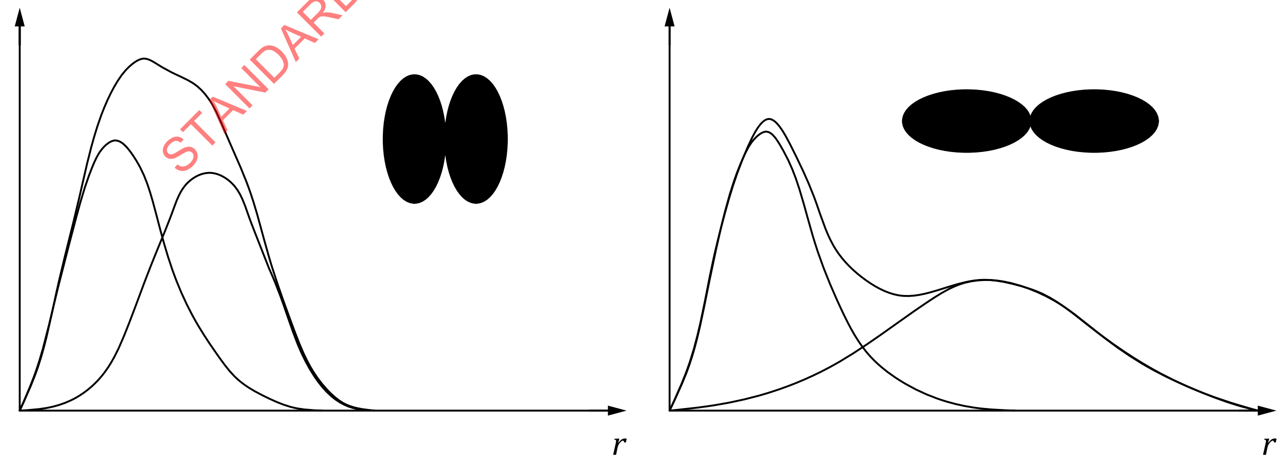


Figure A.5 — The aggregates of two subunits produce PDDF’s that can be recognized by the second peak

Particles of arbitrary or irregular shape produce PDDF's that cannot be analysed without additional information. Any PDDF or scattering function is ambiguous in the presence of polymorphism or significant polydispersity.

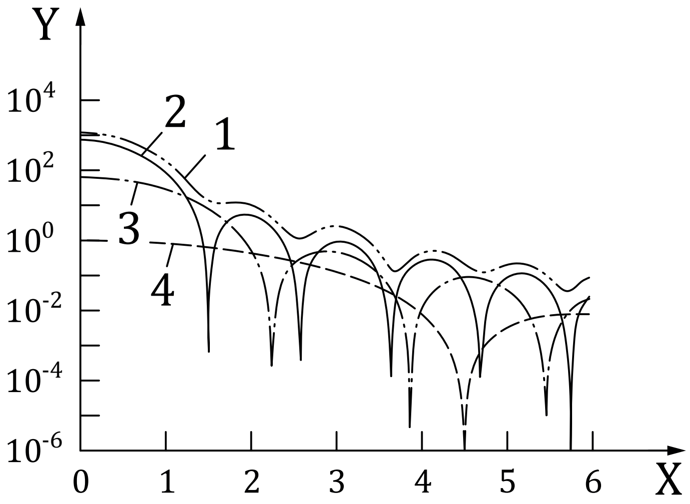
Apart from the method of direct or indirect Fourier transform to real space, the most commonly used method for particle shape analysis is directly fitting experimental data in reciprocal space using an appropriate and pre-selected model. For a dilute monodisperse system containing N uniform particles per unit volume, the interparticle interactions can be neglected, and $I(q)$ depends primarily on the shape and size of the particles.

Therefore, the form factors can be measured experimentally in such systems. The shape and size of monodisperse particles can be obtained by fitting experimental data using functions of form factors. Form factors are known for many elementary shapes such as spheres, cylinders, disks, rods, micelles, lamellas, or Gaussian polymers. For samples with irregular shapes, a concept called 'spherical harmonics'[26] can be used to extract these irregular shapes. For diluted polydisperse system, the size distribution function shall be combined with the form factors for the data fitting. The average size and size distribution can be obtained in this manner. For concentrated systems in which the interactions between the particles are not negligible, the structure factor shall be incorporated for data fitting.

A.6 Polydispersity

There are few truly monodisperse samples. Protein solutions are one example in which all 'particles' can have the same size and shape. There are a number of methods available for determination of a particle size distribution.

The scattering curves of polydisperse or polymorphous samples can be regarded as arising from the sum of all form factors weighted by the respective concentration, squared contrast and volume of the particle. The result of this summation is an averaged form factor which no longer contains sharp minima. Thus a scattering profile showing sharp minima shall arise from an effectively monodisperse material:



Key

1	sum
2	$r = 3$ nm
3	$r = 2$ nm
4	$r = 1$ nm
X	q/nm^{-1}
Y	scattered intensity $I(q)$

Figure A.6 — Effect of polydispersity on the shape of the scattering profile

For a polydisperse system, the scattering function is determined by the shape of the particles and by the size distribution. The polydispersity can be described by a size distribution function, $G(D)$, with a lower cutoff at = 0. The resulting $I(q)$ is given[20][21] by:

$$I(q) = N\Delta\rho^2 \int_0^\infty G(D) V^2(D) P(q,D)\, dD \tag{A.9}$$

where $P(q, D)$ is the normalized form factor of a particle size D. This particular terminology applies only to this International Standard.

There are two ways to compute the size distribution from small angle scattering measurement data $I(q)$:

a) by assuming the analytical form of $G(D)$ and form factor of particle, and then fitting it to the experimental data with least-square fitting optimization of model parameters;

b) by an inverse transformation, the type of transformation depends on the assumed particle shape without assuming the analytical form of $G(D)$.

A number of methods for determining size distribution have been developed in accordance with following these two general methods. The methods were reviewed and are summarized in.[6] The size distribution functions can be assumed as, e.g. Gaussian (normal), log-normal, or Gamma distribution function. The Gamma function can express both a Gaussian-like narrow distribution and lognormal-like wide distribution. Therefore, it can simplify data fitting by selecting one function only. Other methods include Maximum Entropy (MaxEnt) and nonlinear least squares (NLLS).