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Particle size analysis — Small angle X-ray scattering (SAXS)

Analyse granulométrique — Diffusion des rayons X aux petits angles (SAXS)

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Foreword

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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).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 24, *Particle characterization including sieving*, Subcommittee SC 4, *Particle characterization*.

This second edition cancels and replaces the first edition (ISO 17867:2015), which has been technically revised. The main changes compared to the previous edition are as follows:

— inclusion of various methods for the extraction of particle size distribution by using the SAXS method;

— correction of technical terms.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document deals with small-angle X-ray scattering (SAXS), which is performed for particle size analysis in the 1 nm to 100 nm size range. Under certain conditions (narrow size distributions, appropriate instrumental configuration, and idealised shape) the limit of 100 nm can be significantly extended. In ideal circumstances, SAXS can determine the mean particle diameter and particle size 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 interlaboratory 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. SAXS is sensitive to the squared electron density difference. For fixed volume fractions, 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. Core-shell-nanoparticles can also be investigated, but low density (e.g. organic) shells are not detected if the core has a significantly higher density. To obtain the outer particle diameter including the shell, other methods should be used.

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 document 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 needs to be taken and a reduction of the concentration or the addition of salt can 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 document 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 measurable 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 document 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 (SAXS)

1 Scope

This document specifies a method for the application of small-angle X-ray scattering (SAXS) to the estimation of mean particle sizes in the 1 nm to 100 nm size range. It is applicable in dilute dispersions where the interaction and scattering effects between the particles are negligible. This document describes several data evaluation methods: the Guinier approximation, model-based data fitting, Monte-Carlo–based data fitting, the indirect Fourier transform method and the expectation maximization method. The most appropriate evaluation method is intended to be selected by the analyst and stated clearly in the report. While the Guinier approximation only provides an estimate for the mean particle diameter, the other methods also give insight in the particle size distribution.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. 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/TS 80004-2, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 26824, ISO/TS 80004-2 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
particle
minute piece of matter with defined physical boundaries

Note 1 to entry: A physical boundary can also be described as an interface.

Note 2 to entry: A particle can move as a unit.

Note 3 to entry: This general particle definition applies to nano-objects.

[SOURCE: ISO 26824:2013, 1.1]

3.2
particle size
x
d
linear dimension of a particle determined by a specified measurement method and under specified measurement conditions

Note 1 to entry: Different methods of analysis are based on the measurement of different physical properties. Independent of the particle property actually measured, the particle size is reported as a linear dimension, e.g. as the equivalent spherical diameter.

Note 2 to entry: Examples of size descriptors are those based at the opening of a sieve or a statistical diameter, e.g. the Feret diameter, measured by image analysis.

Note 3 to entry: In ISO 9276-1:1998, the symbol x is used to denote the particle size. However, it is recognized that the symbol d is also widely used to designate these values. Therefore the symbol x may be replaced by d.

3.3
radius of gyration
R_g
square root of the ratio of the moment of inertia to the particle mass

Note 1 to entry: Guinier radius (i.e. radius of gyration) is expressed in nanometres. Typical average radii are in the range of 1 nm to 50 nm.

[SOURCE: ISO 26824:2013, 10.2]

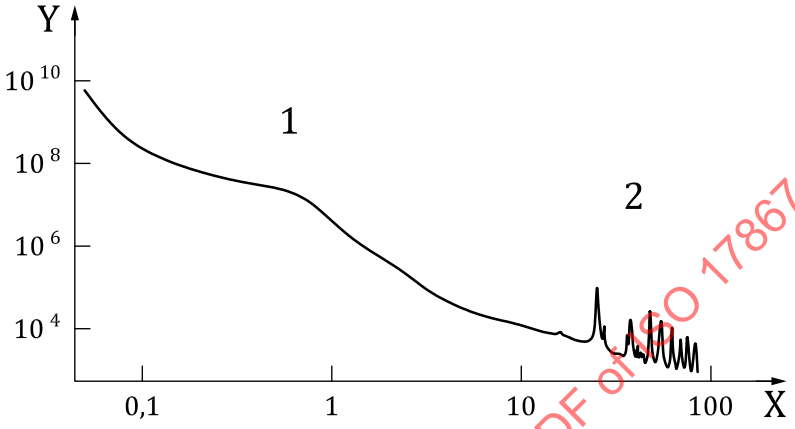
4 Symbols

Symbol	Description	Unit
$\bar{d}_{vs}$	Volume-squared-weighted mean particle diameter	nm
$\bar{d}_{num}$	Number-weighted mean particle diameter	nm
$g_{int}(r)$	Intensity-weighted particle size distribution	
$g_{num}(r)$	Number-weighted particle size distribution	
$g_{vol}(r)$	Volume-weighted particle size distribution	
I_{out}	Primary beam intensity with sample	
I_{in}	Primary beam intensity without sample	
$I(q)$	Scattered intensity (or scattering intensity)	
M	Number of degrees of freedom in fitting	
N	Number of particles	
$P(q, r)$	Particle form factor as functions of q-value and particle radius, r	
q	Momentum transfer or q-value, magnitude of the scattering vector given by $q = 4\pi/\lambda \sin(\theta)$	nm^{-1}
q_{min}	Small angle resolution, minimum accessible q-value	nm^{-1}
q_{max}	Maximum accessible q-value	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	
V	Volume of particle	nm^3
2θ	Scattering angle	deg or rad
λ	Wavelength of the incident X-rays in vacuum	nm
μ	Linear absorption coefficient	mm^{-1}
$\rho(r)$	Electron density distribution	nm^3
σ	Standard deviation of size distribution	nm

5 Principle of the method

When electromagnetic radiation passes through matter, a small fraction of the radiation may be scattered due to electron density differences in the matter. The scattered radiation intensity profile (as a function of the scattering angle or momentum transfer, q), contains information that can be used to deduce morphological characteristics of the material. When X-rays are used to probe a geometrically

ordered group of particles or molecules ("crystals"), the well-known X-ray diffraction pattern is obtained at wide scattering angles, which can be used to characterize the unit cell and lattice constants of such crystalline material. In the small-angle regime (typically $2\theta < 5$ °; wavelength dependent), information on the particle or pore dimensions within the material is available from the elastic scattering arising from the electron density contrast between the particles and the medium in which they reside. This is analogous to static light scattering and small-angle neutron 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

X scattering angle 2θ (in degrees)
Y intensity
1 SAXS range
2 XRD range

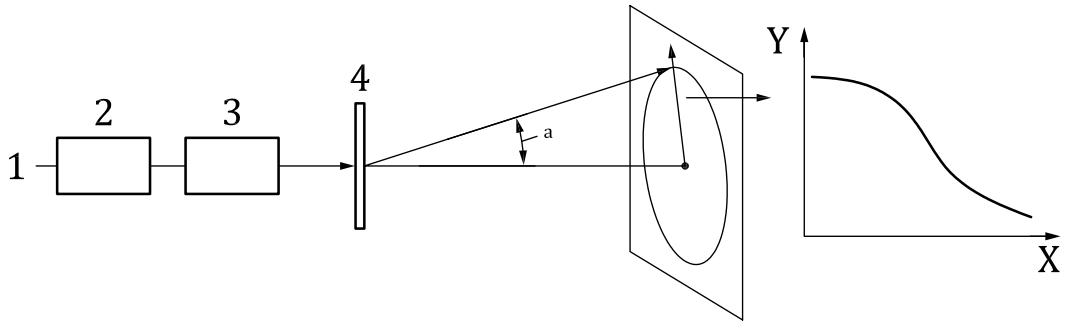
Figure 1 — X-ray scattering diagram illustrating the small-angle SAXS region (left hand side) and the wide-angle XRD (X-ray diffraction) region (right hand side) of a titanium dioxide powder

At low concentration, the small-angle scattering region contains information about the particle morphology, which may be evaluated to extract either the particle size (distribution), or particle shape. Only in very few cases is it possible to obtain both size and shape information. Different regions in q are dominated by signals from a particular length range, and so can contain distinct (exploitable) information aspects. In the low-q range, the Guinier approximation can be applied as an indicative method to get an intensity weighted mean size, provided the particles are smaller than $2\pi/q_{\min}$. Model fitting can be applied in the full range of q to compute a traceable particle size and size distribution with associated uncertainties. Both 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 document. In practice, a concentration ladder may be explored 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 allow identification of 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. In particular, the Guinier approximation is highly sensitive to concentration-induced scattering effects.

6 Apparatus and procedure

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



Key

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

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 unscattered, transmitted beam ("direct beam") from the scattered radiation at small angles (around 0,1°). The direct beam is normally 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).

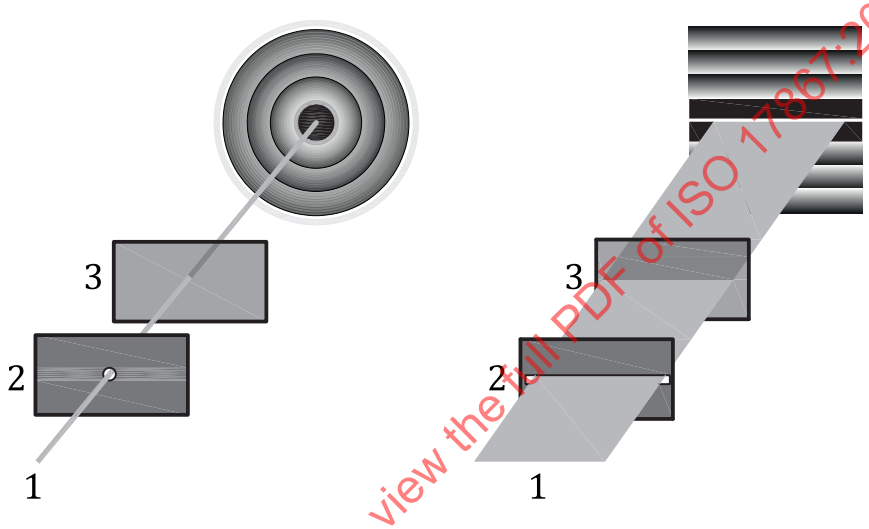
— Point collimation systems have multiple pinholes or crossed slits that limit the shape of the X-ray beam to a low divergence and a small dimension (typically, the beam spot on the sample is less than 0.8 mm in diameter). The scattering is normally centro-symmetrically distributed around the primary X-ray beam. For isotropic samples, the scattering pattern in the detection plane perpendicular to the X-ray beam exhibits circular contour lines around the point of incidence of 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 dimensions can be adapted to accommodate a given sample geometry. 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 reverted using a deconvolution procedure, albeit at the cost of magnified uncertainties of the observed intensities. The investigation of

anisotropic nanostructure with such line-collimated instruments is not as straightforward as for point collimation.

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

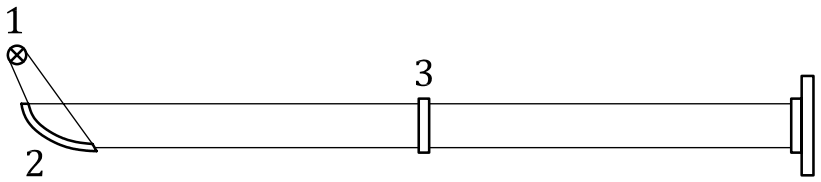
The scattered radiation (containing the morphological information, as described in Clause 5) forms a pattern that contains the information on the size and structure of the sample. This pattern is detected typically by a 1-dimensional or 2-dimensional flat X-ray detector situated behind the sample and perpendicular to the direction of the primary beam. Some multipurpose diffractometers that combine SAXS and diffraction use a scanning point (0-dimensional) detector. Detector classes commonly used include photon-counting and integration type detectors, although in practice, the least problematic/distorted measurements come from high-dynamic range, direct-detection, photon-counting detectors.



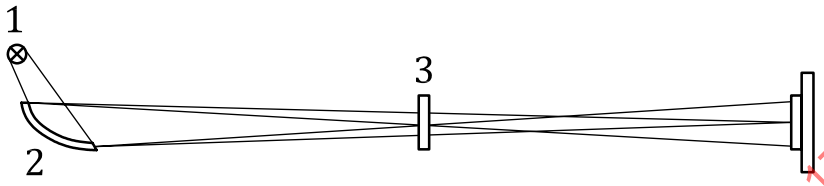
Key

1 X-ray source
2 collimation system
3 sample

Figure 3 — Point and line collimation types used in SAXS



a) Parallel beam



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

Key

1 X-ray source
2 mirror
3 sample

Figure 4 — Focused and parallel beam set-up

7 Preliminary procedures and instrument set-up

Wavelength calibration (see Annex B) can be performed before conducting an experiment and thus would be classified as a preliminary procedure, but this is only required for polychromatic sources. If characteristic X-ray emission lines (e.g. copper Kα or molybdenum Kα lines) are used, a suitable absorber can be used to check that the right emission line has been selected correctly (Nickel for Cu Kα, Zirconium for Mo Kα). Utilization of a calibration material, such as silver behenate, should form part of a full system qualification and fit-for-purpose specification as noted in Annex B.

Only if, in addition to the mean particle diameter and the particle size distribution, the (absolute) concentration or volume fraction of scatterers is to be determined, the intensity shall be scaled to absolute units. For this purpose, a variety of auxiliary calibration materials are available, including water and glassy carbon. Alternatively, some instruments can determine this directly by means of calibrated detectors, or measurement of the unattenuated primary beam intensity on the SAXS detector. The use of semi-transparent beamstops to measure the beam intensity is not recommended due to the radiation hardening effects of such, which can lead to inaccurate values.

All calibrations should be described in the analysis report.

8 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 and can be tuned to optimize the scattering and limit the X-ray absorption, depending on the composition of the sample.

Liquid samples are usually measured inside a thin-walled capillary, the diameter of which is typically between 0,5 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, or higher energy radiation should be used. Viscous samples can be measured better in a paste cell. It is strongly recommended to measure liquid samples in a re-fillable or flow-through container, as this greatly reduces the risks of errors encountered in the background subtraction procedure (incorrigible effects may lead to inaccurate subtraction if non-identical sample containers are used for the two measurements).

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 films, mica or silicon nitride (see Reference [18]). Care should be taken that the scattering from the window material does not affect the result of the measurement and can be appropriately subtracted. For example, polyimide films exhibit a broad small-angle diffraction peak in the vicinity of q approximately 0,7 nm^{-1}, which shall be correctly subtracted in the background subtraction procedure.

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 (see Reference [17]). The optimum thickness, t_0, is given by

$$t_0 = 1/\mu \tag{1}$$

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 pre-treatment (for example, dilution, sonication, annealing or centrifugation) may affect the particle size distribution and should be described in the analysis report.

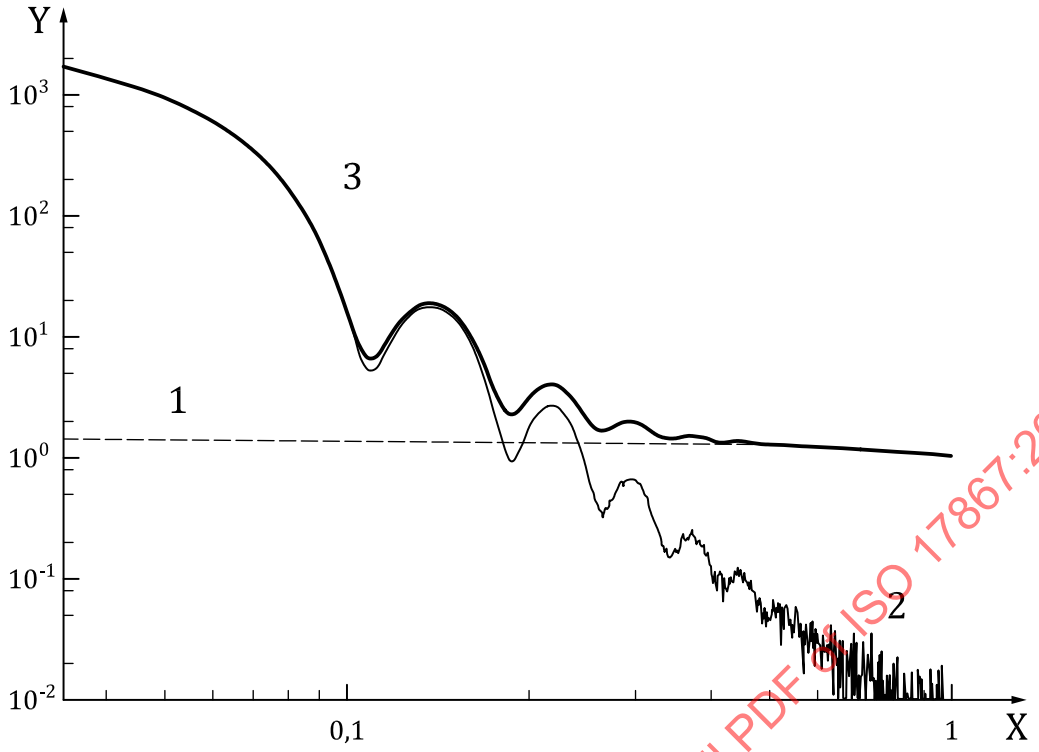
9 Measurement procedure

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

a) a sample measurement (containing signals from the particles, the solvent or matrix, the sample cell windows, the parasitic instrument radiation, background radiation and detector noise);

b) a background measurement (containing signals from the solvent or matrix, the (same) sample cell windows, the parasitic instrument radiation, background radiation and detector noise).

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 shall 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

X q (in nm^{-1})
Y scattered intensity (in a. u.)
1 solvent
2 particle scattering
3 particle dispersion

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.

10 Data correction procedures

As the data analysis methods are sensitive to the quality of the data, the scattering signal of the sample shall be carefully extracted from the total measured signal. These correction steps are performed by the software provided with commercial instruments, or through custom software. At best, they propagate the data uncertainties through the correction steps, resulting in a final data set complete with uncertainty estimates on the data points.

The minimum amount of data corrections to be considered or applied by this software to the data *before background subtraction* are corrections for: invalid pixels, dark counts, time, X-ray flux and X-ray transmission. This is followed by the background subtraction (complying with standard statistics for signals obtained by the subtraction of two independent measurements). After the background subtraction, a normalization for sample thickness can be performed, as well as an optional scaling to absolute intensity units. These corrections may be combined in their implementation, but a discussion of the separate implementation is described in Reference [24]. An overabundance of data points may be reduced using a binning procedure. For size-disperse samples, 100 bins per decade of q are typically sufficient. These bins may be linearly or logarithmically spaced.

For the analysis report, it is sufficient to mention the applied corrections, and the software name and version they have been applied with.

11 Calculation of the mean particle diameter

11.1 General

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

11.2 Guinier approximation

For the Guinier approximation (explained in detail in A.2), $\ln(I)$ 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

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

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 $\bar{x}_{2,6}$.

There are several caveats which concern the validity of this approximation. Firstly, if a straight line is not found in the Guinier region, the approximation does not apply, and no values can be estimated using the Guinier approximation. Secondly, while linearization may be appropriate for on-site review of data, values quoted in an analysis report should be the result of a fit of the Guinier approximation [Formula (3)] to the unlinearized data, at best weighted by the datapoint uncertainty. Thirdly, a linear relationship in the Guinier region is no guarantee for applicability, Data analysis according to 11.3 is required beyond this point, using the Guinier approximation values as an optional guide.

11.3 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:

$$I(q)=N\Delta\rho^2\int_0^\infty P(q,r)\,g_{\text{num}}(r)\,dr+c \tag{6}$$

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:

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

and a lognormal distribution can be written as:

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

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

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

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

According to ISO 9276-2, $\bar{d}_{\text{num}}$ 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.

12 Size distribution determination

12.1 Limitations of size distribution determination from SAXS data

When considering the analysis of scattering patterns with the intent to extract size distributions, there are several limitations to consider. The main limitations include:

a) the upper and lower size bounds, as defined by the data limits;

b) the practical necessity for uncertainty estimates on the data;

c) the required assumptions on the scatterer shape and (in some cases) the mathematical form of the size distribution; and

d) the inherent size-weighting of information in the SAXS data. These limitations will be briefly addressed.

The range of dimensions that can be probed with SAXS is in general limited by the measurement range (a second, less common limitation to the upper limit in size is given by the transversal coherence length of the radiation). The maximum and minimum probed dimensions are defined in Formula (A.3). Information obtained beyond these limits may not be grounded in actual data, but rather will be present due to the assumptions made.

In the data fitting procedures described herein, the agreement between the model and the measured data are normalized by the uncertainty estimates of the measured data values. While it is technically possible to bypass the normalization, such a circumvention is strongly discouraged as it can easily lead to overfitting and misinterpretation (skewed weighting) of the information. The normalization by the uncertainty ensures that the data are weighted by the accuracy of each individual data point, and furthermore helps prevent overfitting due to its provision of a clear cut-off criterion.

Due to the information loss inherent in the process of small-angle scattering, additional external information shall be provided in order to arrive at a unique solution. The information required to obtain a size distribution includes a shape assumption (globular by default), an assumption on the inter-scatterer interaction (dilute, i.e. no interaction by default), and, for one of the methods, an assumption on the mathematical form of the size distribution (typically log-normal, Gaussian or Schultz-Zimm). This information can be either assumed (on the basis of reasonable expectations) or provided by external methods (e.g. microscopic methods). For mixtures of scatterers differing in shape, very strict assumptions (or rather provision of external information) may still allow an arrival at the correct solution.

The exact contribution is dependent on the shape of each scatterer, the measurement range, and the angle-dependent collection efficiency of the instrument. The overall effect, however, is that the detection limits of small scatterers deteriorates with increasing presence of large scatterers. For spherical particles with homogeneous electron density and narrow size distribution, the volume-weighted and the number-weighted size distribution can be obtained. For other particles, the number-weighted size distribution is not easily accessible without additional assumptions (see Reference [25]).

12.2 A brief overview of methods

The methods are separated into model-based and non model-based methods. The model-based data fitting is the easiest to implement but requires the assumption both the scatterer shape as well as the mathematical form of the distribution. The non model-based methods can derive the distribution form-free, requiring only the assumption on the general shape of the scatterer.

12.3 Goodness of fit: evaluating fits

For all fitting methods, the agreement between the data [$I_{\mathrm{meas}}(q)$] and the model [$I_{\mathrm{model}}(q)$] can be evaluated using the reduced chi-squared value (χ_r^2). This value is large for a large discrepancy between the two, but approaches unity when the model fits the data on average to within the uncertainty [$\sigma(q_i)$] of the data. For most data fitting methods, the reduced chi-squared value is used in the optimization procedure directly. The calculation of χ_r^2 follows:

$$\chi_r^2 = \frac{1}{N_{\mathrm{d}} - M} \sum_{i=1}^{N_{\mathrm{d}}} \left[\frac{\mathrm{I}_{\mathrm{meas}}(q_i) - I_{\mathrm{model}}(q_i)}{\sigma(q_i)} \right]^2 \tag{11}$$

where N_{d} denotes the number of data points, M denotes the number of degrees of freedom in the fitting procedure, and i denotes the index of a given data point.

12.4 Model-based data fitting

In the model-based data fitting procedure, the goodness-of-fit value is minimised (and thereby the agreement between model and data maximized) by adjusting a limited number of parameters of a model function. One common model function describing the scattering of a sample containing size-disperse scatterers is given in Formula (6), combining the form factor of a particularly shaped scatterer with a

monomodal, single-parameter size distribution. This equation is evaluated numerically to compute the model scattering function.

There are several minimization methods available to find the model parameters that minimize the goodness-of-fit. The most common of these is the Levenberg-Marquardt nonlinear least-squares solver, for which libraries are readily available. This minimization method furthermore provides uncertainties on the final values of the (minimised) fit parameters. However, it may become unstable when models are fitted with many parameters in complex models. For this reason, modern, more stable adaptations of the Levenberg-Marquardt methods should be implemented where possible.

The model-based data fitting procedure is the most commonly implemented data analysis method in SAXS and can be found in a wide variety of free and open-source data fitting software programs. These programs differentiate mostly in the datatypes they can read, and the models that are implemented therein. However, each of these programs requires the selection of several assumptions.

Firstly, the scatterer shape model needs to be selected. The choice of model is ideally driven by information from complementary methods. Secondly, the variable dimension needs to be assigned to a particular form of the distribution (often, the form can be assumed based on the formation process of the scatterers). A fitting range and variable constraints can optionally be added.

Starting parameters can lead the minimization method to a local minimum rather than a global minimum, and a variety of starting parameters needs to be chosen to ensure a global minimum is reached. As for uniqueness, any number of combinations of shape and distribution may fit a measured scattering pattern, and it is tempting to assume that the combination leading to the best fit is correct. It is therefore important to realize that it is theoretically impossible to retrieve information on both shape as well as the mathematical form of the distribution. One of these two parameters shall therefore be given and supporting information shall be provided on why this choice was made.

12.5 Monte Carlo-based data fitting

A Monte Carlo based data fitting procedure can be used to remove the requirement to select a mathematical form of the size distribution. Using solely information on the elementary scatterer shape, it can retrieve any size distribution within the size limits dictated by the measurement. These procedures are therefore less reliant on assumptions and are more data-driven.

This procedure relies heavily on the goodness of fit measure and is therefore reliant on the provision of representative uncertainty estimates. Furthermore, since no assumption is made on the asymptotic behaviour of the size distribution, extrema of the size distribution carry a higher uncertainty than the central component. Lastly, since the representation of scatterers in scattering data are not proportional to their amount, but weighted proportional to their size, Monte Carlo methods are often unable to positively confirm the existence of small numbers of small scatterers in the presence of larger scatterers. They are best used to determine volume-weighted distributions, which closely approaches the information content in the measured data.

In such procedure, the model intensity is described as a summation of many individual contributions (with each contribution typically the form factor of a single scatterer). In each Monte Carlo iteration, a single contribution of the set is replaced by a newly calculated one. A parameter (typically a size) of the newly calculated contribution is allowed to vary randomly. If such a replacement leads to a reduction of the reduced chi-squared value, the replacement is kept, otherwise reverted. Through such trial-and-error replacements, a combination of contributions can be arrived at, which describes the scattering intensity to within the uncertainty of the procedure (i.e. when the condition $\chi_r^2 \leq 1$ is reached). Through analysis of the spread of the values of the varying parameter, a distribution can be approximated.

An uncertainty on the resulting size distribution can be obtained by repeating the optimization procedure multiple times. A successful optimization will result in similar results for every independent repetition. If all results are distinctly different, then the data does not support extraction of the information on the attempted parameter.

12.6 Indirect Fourier transform (IFT) method

In the indirect Fourier transform (IFT) based method the scattered intensity is assumed to result from an ensemble of particles of the same shape (see Reference [12]). The relation between the size distribution, more precisely the number-weighted distribution of the particles $g_{\mathrm{num}}(r)$ and the experimental scattering intensity *I(q)* is already given in Formula (6).

The IFT method estimates the size distribution by a general approach, the shape of the particles is assumed to be known a priori. By default, spherical particles are assumed for determining the size distribution, however, other form factors can be applied for evaluation of prolate or flat particles. For calculating the size distribution, basic functions (cubic B-splines) are subjected to the scattering function $I(q)$ involving stabilization condition minimizing and weighted least square approximation to the experimental data. Apart from the number-weighted distribution $g_{\mathrm{num}}(r)$, it is common to determine the intensity- and volume-weighted distributions $g_{\mathrm{int}}(r)$ and $g_{\mathrm{vol}}(r)$ of the dispersed particles. The three types of distributions are related to each other by $g_{\mathrm{int}}(r) \propto g_{\mathrm{vol}}(r) r^3 \propto g_{\mathrm{num}}(r) r^6$.

In a scattering experiment, intensities are recorded, so calculating the intensity-weighted distribution depicts how much intensity is caused by the individual particles (scatterers). If the analysed sample consists of a majority of small particles and a minority of a few big ones, most of the scattering intensity will be caused by the few big particles (due to the r^6 scaling). The low scattering intensity caused by the small particles does not reveal their actual number well in an intensity-weighted distribution. Finally, the volume-weighted distribution illustrates in principle which volume (concentration) the particles occupy within the irradiated sample volume.

12.7 Expectation maximization (EM) method

The expectation maximization (EM) method (see Reference [5]) is an algorithm that determines the size distribution by seeking the log-likelihood maximum for these parameters using the experimental scattering data. When applied to the small angle scattering of nanoparticles, EM provides the particle size distribution offering the highest level of agreement – from the statistical point of view – with the experimental data (see Reference [4]). Like the Monte Carlo-based data fitting, EM makes no assumptions on the mathematical form of the distribution and the only required user input for the shape of the elementary scatterer.

The size distribution is described on a grid complying with the Nyquist-Shannon constraints (see Reference [32]) imposed by the data sampling and the maximum of q-values.

One of the most important features of the EM method compared to alternative model-free fitting procedures is the fact that it exploits the Poisson statistics of the scattering data, which can be accurately represented using a photon counting detector. For normalized intensity profiles, the Poisson statistics are often lost through azimuthal averaging and normalization and therefore are first restored, on the basis of experimental normalized intensities and uncertainties, before applying the algorithm.

The search for the size distribution is performed within an iterative process, which is formally guaranteed to converge (see Reference [4]). The size distribution is initialized with a constant function, then updated at each iteration based on a rule deduced from the optimality criterion (see Reference [4]). Like the Monte Carlo-based methods, the model intensity is described as the summation of contributions from individual scatterers, weighted by the size distribution. The iterative process is stopped when the difference between the data and the model intensity is within the expected noise level in the manner of a Morozov discrepancy principle (see Reference [21]).

The EM-based analysis outputs are the size distributions (either number- or volume-weighted) as well as the corresponding visibility limit, i.e. the minimum population at a given size that may non-equivocally be extracted from the data, given the Poisson uncertainty.

Since EM is formally guaranteed to converge, results are strictly reproducible for a given set on input data. This excludes the possibility to estimate uncertainties in the manner of Monte Carlo-based methods. However, uncertainty estimates can be generated by running a set of independent EM calculations whose input is the model intensity of the exact solution with randomly added Poisson noise.

13 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 calibration of the q-axis can be validated for each individual instrument with a suitable calibration standard. A frequently used material is silver behenate.

14 Documentation and test report

14.1 Test report

Test reports should be prepared in accordance with ISO 9276-1 [37] and ISO 9276-2 [38] and shall contain at least the following information.

a) A reference to this document.

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 [39] 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:

$$u_{Rg} = -3\left(u_{slope}\right)/\left(2R_g\right) \tag{12}$$

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 [38].

d) The 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, model-based data fitting, Monte Carlo-based data fitting, indirect Fourier transform (IFT) method or expectation maximization (EM) method].

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.

14.2 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 [40]. 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 principle

A.1 Data interpretation

The observed, corrected scattered intensity for a dispersed, non-interacting analyte is related to the particle scattering through:

$$I_{\text{particle}}(q)=N|F(q)|^2 \tag{A.1}$$

where the scattering amplitude, $F(q)$, is defined as follows:

$$F(q)=\int_V \Delta\rho(r)e^{\text{iqr}}dV \tag{A.2}$$

It depends on the particle shape. The electron density contrast, $\Delta\rho(r)$, is the difference between the scattering length electron densities of the scatter and the matrix, and V is the volume of the particle.[14] The form factor $P(q)=|F(q)|^2$ contains information on electron density distribution and shape of the particle.

Ideally, resolution is tested with an appropriate reference material or mixtures of known and fully documented materials. The limitations are governed by the wavelength of the utilized radiation and the range of scattering angles between which the scattering pattern is reliably sampled, as well as the sampling frequency. While the upper size limit is strictly determined by the sampling resolution $\min(\Delta q)$ between which non-correlated signals can be extracted, in practice (i.e. when the beamstop size roughly matches the dimensions of the primary beam), the following approximations are appropriate.

A scattering profile measured between $q_{\min}$ and $q_{\max}$ can be used to resolve particle features with length scale between $d_{\min}$ and $d_{\max}$ when:

$$d_{\min}=2\pi/q_{\max} \text{ and } d_{\max}=2\pi/q_{\min} \tag{A.3}$$

where $q = 4\pi/\lambda \sin(\theta)$ is the momentum transfer defined by the wavelength of the X-rays and the 2θ scattering angle defined by the direction of the incident beam and the viewing direction of the detector.

The challenge is to reach a small $q_{\min}$ without measuring the intense direct beam. The beam size, the quality of the collimation system, and the alignment of the beam stop are the main factors that determine the possible $q_{\min}$.

In general, there are three possible notable influences on the data from the used SAXS instrument: beam-dimensions (beam length and beam width), detector cross-talk (point-spread functions) and wavelength distributions.

With respect to beam dimension, for point-collimation instruments desmearing of the data are normally not required (assuming an ideal round, non-elongated point). Therefore, the experimental data can be directly used for data modelling or Guinier's analysis.

When using line-collimation instruments, care has to be taken to account for the presence of instrumental broadening, i.e. the line-collimated beam. For evaluating experimental data measured in line collimation it is 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, however, in this document the second method is recommended as it is more reliable.

Small-angle neutron scattering is not included in this document 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.2 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.

$$R_g^2 = \frac{\int_v r^2 \rho(r) dV}{\int_v \rho(r) dV} \tag{A.4}$$

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

$$p(r) = \left\langle \int_V \Delta\rho(\vec{s}) \Delta\rho(\vec{s}-\vec{r}) \overrightarrow{ds} \right\rangle \tag{A.5}$$

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:

$$R_g = (3/5)^{1/2} r \tag{A.6}$$

where *r* is the radius of a homogeneous spherical particle.

Thus, radii of gyration shall be multiplied by (5/3)1/2 ≈ 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:

$$R_g = \sqrt{R^2/2 + L^2/12} \tag{A.7}$$

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

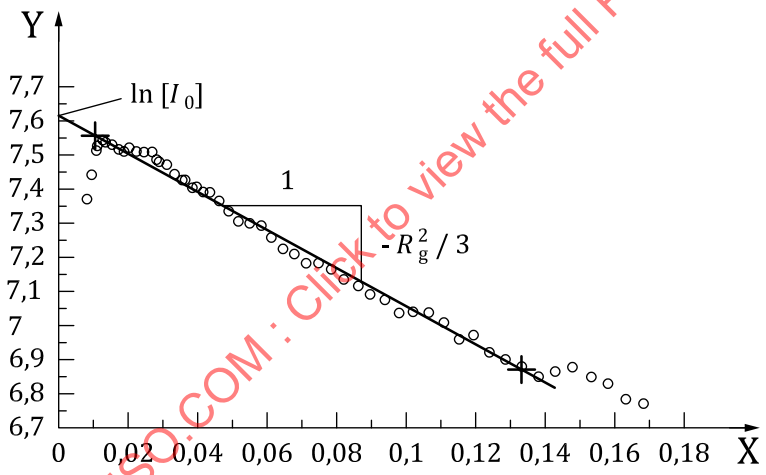
$$I(q) = I_0 \exp\left[-\frac{1}{3}R_g^2 q^2\right] \tag{A.8}$$

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.8) is used in its logarithmic form, leading to the linear equation:

$$\ln[I(q)] = \ln[I_0] - \frac{1}{3}R_g^2 q^2 \tag{A.9}$$

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 (in 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:

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

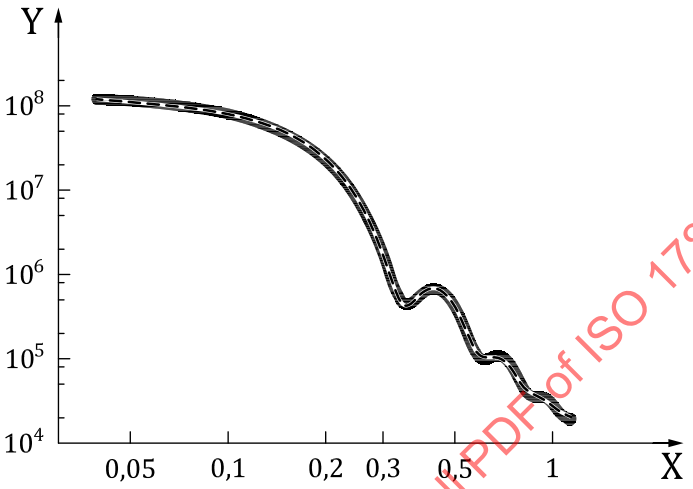
Thus, R_g is 4,0 nm.

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 needs to be performed using the other methods in this document.

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.3 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 particles, only the radius determines the period of the oscillations which are observed for sufficiently monodisperse particles. The amplitude of the oscillations is decreased with increasing 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.[20]



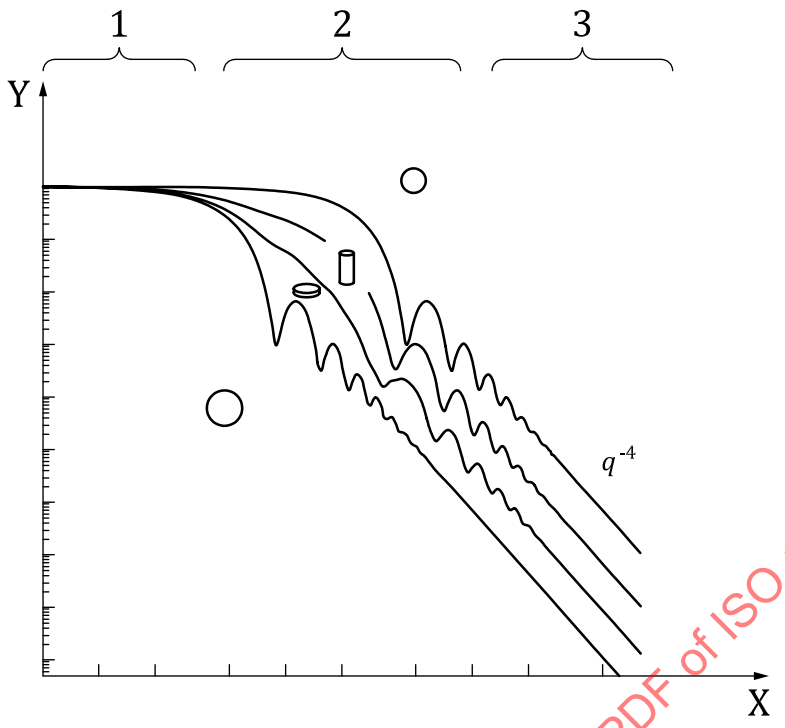
Key

X		q (in nm^{-1})
Y		scattered intensity $I(q)$
	▬▬	measured
	- - - - - - -	fit

Figure A.2 — Measured scattered intensity and fit using the form factor for homogeneous spheres with a Gaussian size distribution (from Reference [19])

A.4 Particle shape

Every particle can be described by a form factor that is characteristic of the structure of the particle. Accurate shape determination from SAXS data are possible for very narrow size distributions. The slope of the form factor at small angles (where $qR_g < 1$) is primarily determined by the overall size (Guinier region) and the final slope at large angles is indicative of the surface (Porod region). The oscillating part in the middle section of the form factor (Central or Fourier region) bears additional information on the shape and the internal electron density distribution:



Key

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

Figure A.3 — Information domains of a particle form factor are Guinier, Fourier, and Porod regions (double-logarithmic plot)

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 –1 or –2 indicates 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. Fourier region also is called as central, crossover, shape regions (see References [10] and [13]).

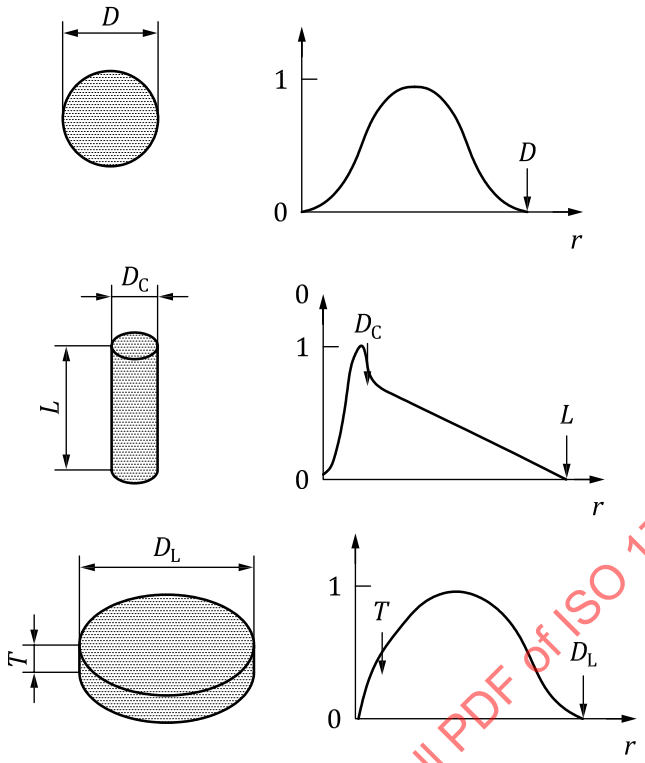
By identifying key features in the PDDF, the shape of a particle can be quickly classified into

a) spherical or globular;

b) prolate or cylindrical; and

c) oblate or lamellar symmetry.

Three typical cases listed above are shown in Figure A.4.

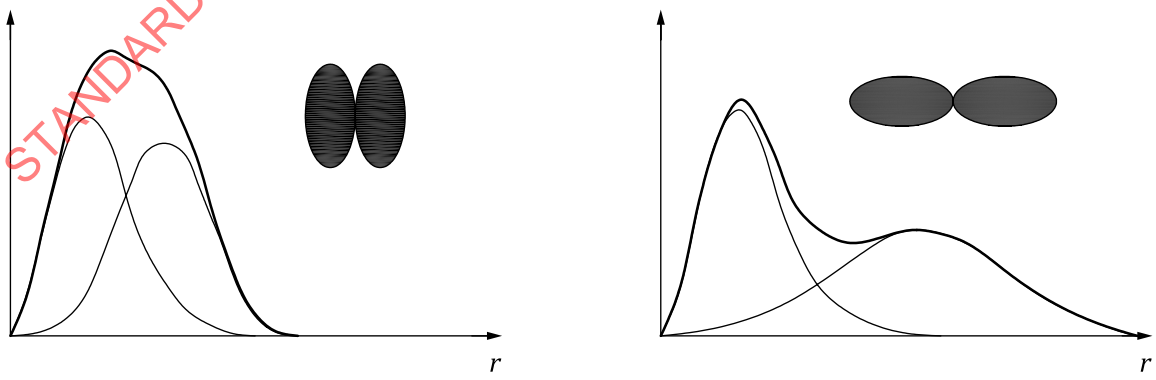


Key

D maximum length of the objects, diameter of spherical object
D_c section diameter of the elongated object
L length of the elongated object
D_L diameter of the oblate object
T thickness of the oblate object
r distance from the centroid of the object

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' arrangements as these will show a secondary peak as in Figure A.5.



Key

r distance from the object centroid

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