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Determination of particle concentration by small-angle X-ray scattering (SAXS)

Détermination de la concentration de particules par diffusion des rayons X aux petits angles (SAXS)

Reference number
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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).

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

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

Small-angle X-ray scattering (SAXS) is a well-established method to obtain structural information on inhomogeneities in materials at the nanoscale, typically between 1 nm and 100 nm, and is thus perfectly suited for nanoparticulate systems. Under certain conditions, the upper limit can be extended to 200 nm and beyond. For sufficiently monodisperse spherical particles, the observed oscillations of the scattered intensity as a function of the momentum transfer, which is directly related to the scattering angle and the wavelength of the incident X-rays, enable the size determination of nanoparticles. In order to determine their concentration in a liquid (also called suspending medium, solvent or matrix), the absolute differential scattering cross section has to be determined, thus the ratio of the scattered intensity to the incident intensity. Assumptions on the particle shape are required, which can be based on microscopic techniques like electron microscopy. Furthermore, the electron density difference between the particles and the liquid needs to be known.

The concentration of nanoparticles, thus particles in the size range between about 1 nm to 100 nm, is one of the most important parameters for nanoparticle use in industry, medicine and research, and is expected to become relevant as well for regulatory purposes, especially in the pharmaceutic sector. The application of SAXS for the determination of the mean particle size and size distribution has been described in ISO 17867. This document covers the extension to obtain the nanoparticle concentration as well from SAXS measurements. User-friendly commercial SAXS instruments are available worldwide from several 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 a document that allows users to obtain good inter-laboratory agreement on the accuracy and reproducibility of the technique.

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.

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Determination of particle concentration by small-angle X-ray scattering (SAXS)

1 Scope

This document deals with the application of small-angle X-ray scattering (SAXS) for the measurement of the particle concentration in suspensions. In this document, only the concentration of sufficiently monodisperse spherical particles is treated, which means that the width of the size distribution is typically below about 50 % of the mean diameter. Here, the differential scattering cross section can be calculated based on the form factor, which depends only on the momentum transfer q and the particle radius r. Furthermore, this document is limited to 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 can be necessary.

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 17867, *Particle size analysis — Small angle X-ray scattering (SAXS)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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

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

— IEC Electropedia: available at https://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 definition applies to nano-objects.

[SOURCE: ISO/TS 80004-6:2021, 3.9]

3.2
particle size
linear dimension of a *particle* (3.1) 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 can be reported as a linear dimension, e.g. as an equivalent spherical diameter.

[SOURCE: ISO/TS 80004-6:2021, 4.1.1]

3.3
particle size distribution
distribution of *particles* (3.1) as a function of *particle size* (3.2)

Note 1 to entry: Particle size distribution may be expressed as cumulative distribution or a distribution density (distribution of the fraction of material in a size class, divided by the width of that class).

[SOURCE: ISO/TS 80004-6:2021, 4.1.2]

3.4
suspension
heterogeneous mixture of materials comprising a liquid and a finely dispersed solid material

[SOURCE: ISO/TS 80004-6:2021, 3.13]

3.5
concentration
amount-of-substance of a component divided by the volume of the system

[SOURCE: ISO 18113-1:2022, 3.2.12]

3.6
particle number concentration
number of particles per unit of volume of suspension

Note 1 to entry: The particle number concentration can also be given as number of particles per unit of mass of suspension. Literature values for the density of the liquid can be used for the conversion as, in most cases, the low content of particles for which this document is applicable will not affect the sample density significantly.

[SOURCE: ISO 29464:2017, 3.2.131]

4 Symbols and abbreviations

The symbols and abbreviations used in this document are listed in Table 1.

Table 1 — Symbols

Symbol	Description	Unit (with prefix)
C	Particle number concentration	l^{-1}
$\bar{d}_{ln}$	Median of lognormal size distribution	nm
$\bar{d}_{num}$	Number-weighted mean particle diameter	nm
f_1, f_2	Atomic scattering factors	
$g_{num}(r)$	Number-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	Molar mass	g/mol
N	Number of particles	
N_A	Avogadro constant	mol^{-1}
$P(q, r)$	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}	Minimum accessible q-value	nm^{-1}
q_{max}	Maximum accessible q-value	nm^{-1}
r	Particle radius	nm

Table 1 *(continued)*

Symbol	Description	Unit (with prefix)
r_e	Thomson radius	fm
$S(q,r)$	Structure factor as functions of q-value and particle radius, r	
T	Transmission	
t_o	Optimum sample thickness	mm
V	Volume of particle	nm^3
w	Sample thickness	mm
Z	Number of protons	
λ	Wavelength of the incident X-rays in vacuum	nm
μ	Linear absorption coefficient	mm^{-1}
ρ	Mass density	g/cm^3
ρ_e	Electron density	nm^{-3}
ρ_{e_P}	Electron density of particles	nm^{-3}
ρ_{e_L}	Electron density of the liquid	nm^{-3}
$\Delta\rho_e$	Electron density difference	nm^{-3}
σ	Standard deviation of Gaussian size distribution	nm
$\dfrac{d\Sigma}{d\Omega}(q)$	Differential scattering cross section per volume	$cm^{-1} sr^{-1}$
σ_{ln}	Standard deviation of logarithm of particle size distribution	
2θ	Scattering angle	deg or rad

5   Principle of the method

When X-rays pass through matter, a small fraction of the radiation can 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. In the small-angle regime (typically $2\theta < 5°$; wavelength dependent), information on the particle 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, typically a liquid. For sufficiently monodisperse spherical nanoparticles, the scattering pattern consist of concentric rings, corresponding to oscillations of the scattered intensity as function of the scattering angle or momentum transfer, q. If the (electron) density of the particles and the surrounding liquid are known, the nanoparticle concentration can be determined by comparing the calculated and the measured differential scattering cross section. The method requires the calibration of the q-axis and the intensity axis. The absolute scattering cross section can be obtained by using either:

— primary or secondary standards such as water, Lupolen or glassy carbon with calculable or known scattering cross section;

— an area detector with very high dynamic range (such as hybrid-pixel detectors) so that the incident radiation (direct beam) and the scattered radiation can be measured;

— an area detector with known quantum efficiency for the scattered radiation and an additional detector (such as a calibrated photodiode) to determine the incident photon flux.

Calibration with reference materials consisting of nanoparticles with known concentration is not required in these cases.

If an absolute intensity calibration is not possible, it is still possible to determine the mass concentration from the extrapolated forward scattering intensity using a similar monodisperse reference material with known mass concentration.

At increased concentrations, i.e. those higher than ten 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 this 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.

## 5.1 Particle size detection limits

The determination of the particle size (mean particle diameter) and the size distribution shall be in accordance with ISO 17867. The accessible size range strongly depend on the instrument. In order to register at least one minimum in the scattered intensity as function of the momentum transfer, the lower diameter limit is typically a few nm. The higher diameter limit is about hundred nm for most laboratory instruments, but the range can be extended to several hundred nm at dedicated ultra small angle X-ray scattering (USAXS) instruments which are available at some synchrotron radiation facilities and some laboratory instruments.

5.2 Particle concentration detection limits

The particle number concentration limits vary as well with the instrument, but even more with the size and the (electron) density of the particles. The scattered intensity of spherical particles scales with the sixth power of the particle size, thus the accessible lower number concentration limit of large particles is orders of magnitude lower. On the other hand, the scattered intensity scales with the square of the electron density difference between particles and liquid. Therefore, much lower concentration ranges are accessible for gold nanoparticles compared to polystyrene particles. Table 2 provides typical orders of magnitude for the lower limit of detection (LLD) for nanoparticles of different diameters and materials suspended in water. The accessible concentration ranges are schematically shown in Figure 1.

Table 2 — Lower limit of detection for nanoparticle number concentration

Material	Density g/cm^3	Diameter nm	LLD number concentration l^{-1}
Polystyrene	1,05	10	10^{20}
Polystyrene	1,05	100	10^{17}
Silica	2,65	10	10^{17}
Silica	2,65	100	10^{14}
Silver	10,49	10	10^{15}
Silver	10,49	100	10^{12}
Gold	19,28	10	10^{14}
Gold	19,28	100	10^{11}
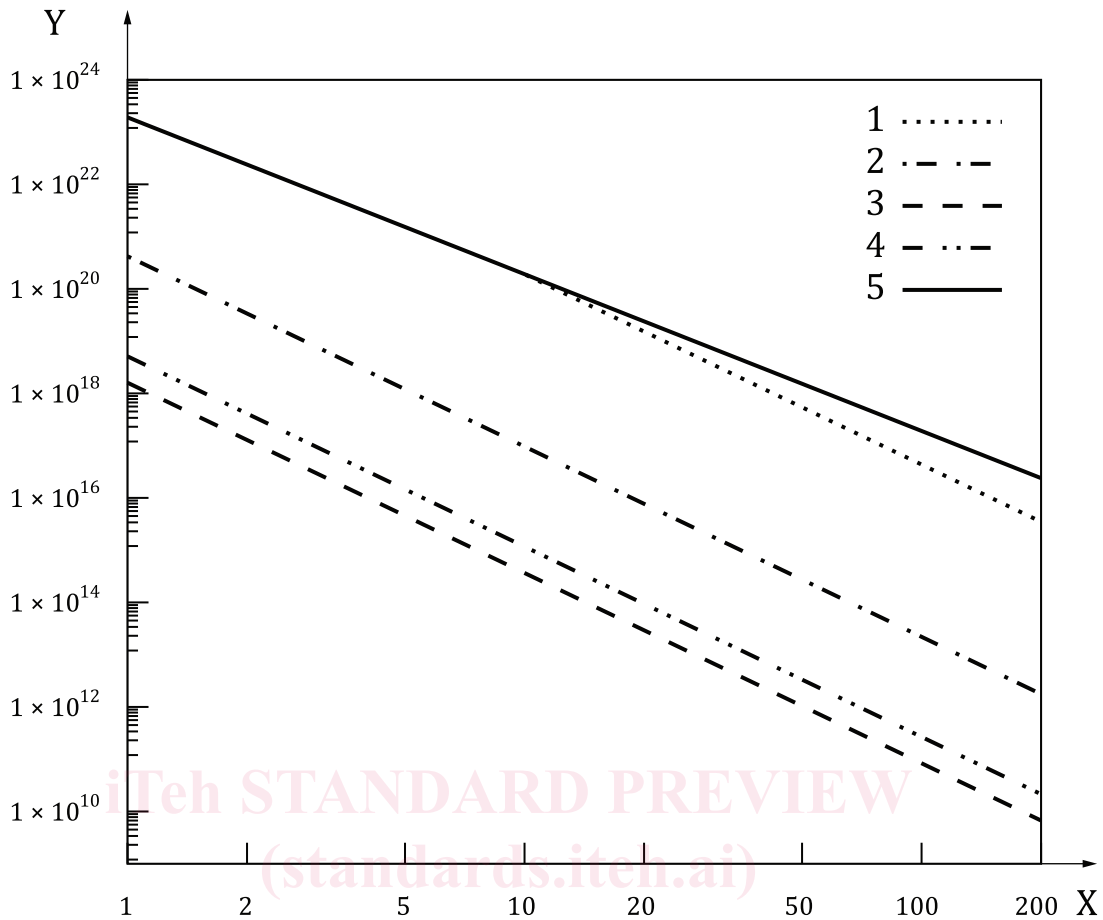
Key

X   diameter/nm
Y   number of particles/litre
1   lower limit for polystyrene particles in water
2   lower limit for silica particles in water
3   lower limit for gold particles in water
4   lower limit for silver particles in water
5   upper limit for all particles corresponding to 10 % volume fraction

NOTE   For low-density particles like polystyrene, the low concentration limit can be decreased, for example, by using ethanol instead of water as liquid.

Figure 1 — Schematic representation of the accessible concentration ranges for spherical nanoparticles of four different materials (gold, silver, silica and polystyrene) in aqueous suspension as function of the particle diameter

5.3   Effects of polydispersity

The issue of polydispersity is extremely important for most real samples. As mentioned above, the scattered intensity is proportional to the sixth power of the radius, thus the scattering from larger particles can hamper the detection of smaller size fractions in poly-dispersed samples. Thus, the samples have to be sufficiently monodisperse. If the size distribution is too broad, minima are no longer observed in the scattering curve, and thus a unique model fitting is no longer possible. Also, a size distribution asymmetry, thus a non-Gaussian or non-lognormal size distribution would lead to deviations for the particle concentration.