



# EMPIR



The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

Grant Agreement number	17NRM04
Project short name	nPSize
Project full title	Improved traceability chain of nanoparticle size measurements
Deliverable Reference number	<b>Deliverable 4</b>
Deliverable Title	<b>Report on uncertainty statements for the nanoparticle concentration determination by SAXS</b>
Lead partner for the Deliverable	CEA
Due Date for Deliverable	June 2021
Actual Date of Submission	October 2021
Coordinator	<b>Dr Vasile-Dan Hodoroaba</b> BAM Tel: +40 30 8104 3144 E-mail: Dan.Hodoroaba@bam.de
Project website address	<a href="https://www.bam.de/Navigation/EN/Topics/Materials/Nanotechnology/nanotechnology.html">https://www.bam.de/Navigation/EN/Topics/Materials/Nanotechnology/nanotechnology.html</a>
Partners	<b>CEA, BAM, PTB</b>

## 1. Introduction

The main objective of the nPSize project is to improve the capabilities for nanoparticle size measurements via methods such as Microscopy and Small-Angle X-ray Scattering (SAXS), which are traceable to SI units. The principle of metrological nanoparticle size determination has been established in several papers <sup>a,b,c</sup> and projects<sup>d</sup>. Work on concentration measurements has also been done<sup>e,c</sup>. In this report, we determine the uncertainties that occur from the calculation of nanoparticle concentrations by SAXS.

## 2. Small-Angle X-Ray Scattering (SAXS)

SAXS is a powerful method for the characterization of suspended nanoparticles, with regard to particles shape, size distribution, or concentration. "Small-Angle" means that photons are scattered at small-angles relative to the direction of the incident photon beam. It is a non-destructive ensemble-method that requires very little sample preparation.

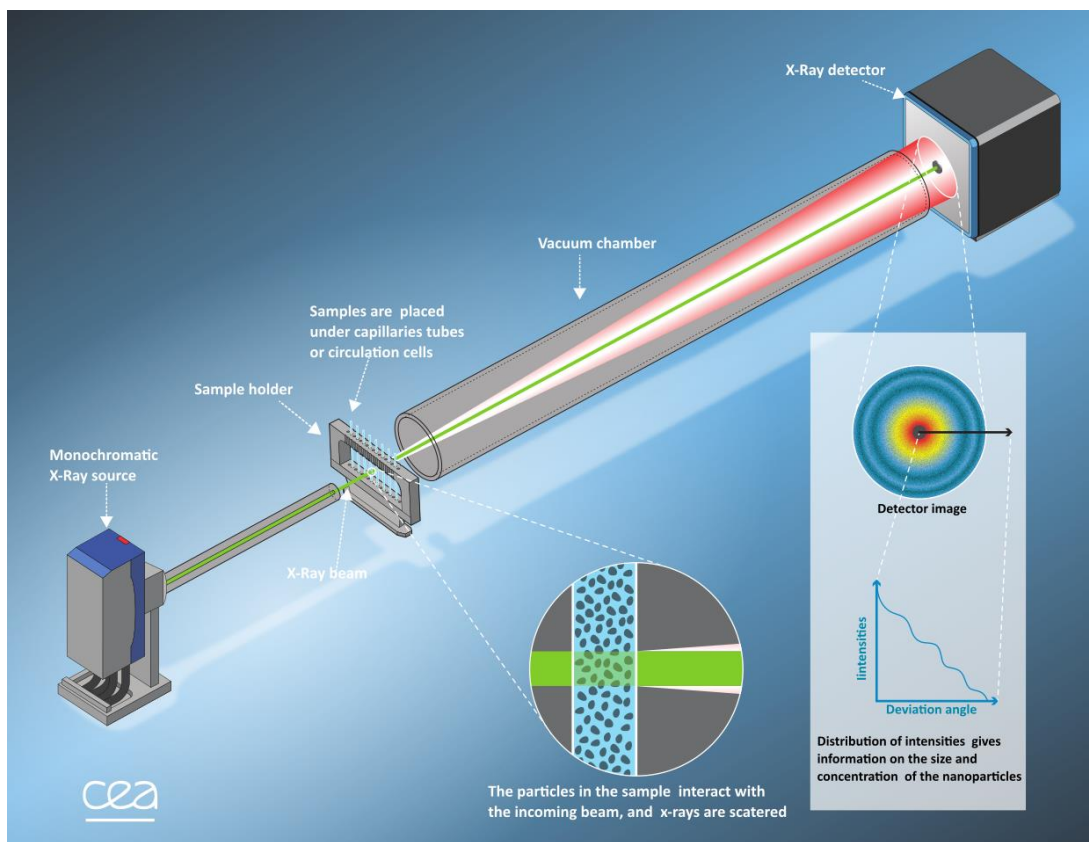


Figure 1 : Principle of SAXS experiment

Generally, the nanoparticle suspensions of interest are filled into capillaries or flow through circulation system and illuminated by x-rays.

At the **PTB**<sup>f</sup>, bending magnets generate synchrotron radiation, which is monochromatized, and then guided and focused onto the sample. The monochromator allows photon energies in the range of 1.75 keV to 10 keV. At the **BAM** laboratory<sup>b</sup> and **CEA** laboratory<sup>g</sup>, commercial X-ray generators provide 8 keV or 17 keV energies. The scattered photons are detected by a detector with the 2D detection plane perpendicular to the incident beam. The X-ray beam path, between the source and the detector, may be in vacuum or partially in the air depending on the instrument.

### 3. Principles of Nanoparticle Size & Concentration measurements

#### 3.1 Scattering Intensity measurements

To determine the diameter size, diameter distribution and number concentration of suspended nanoparticles, the particle suspension is irradiated with X-ray radiation of wavelength  $\lambda$  and photon flux  $\phi_{in}$ . The X-ray photons are elastically scattered by the sample (e.g. suspended particles in a suspension medium in a glass capillary) which is described by the momentum transfer  $\vec{q}$  of the photons during the scattering process.

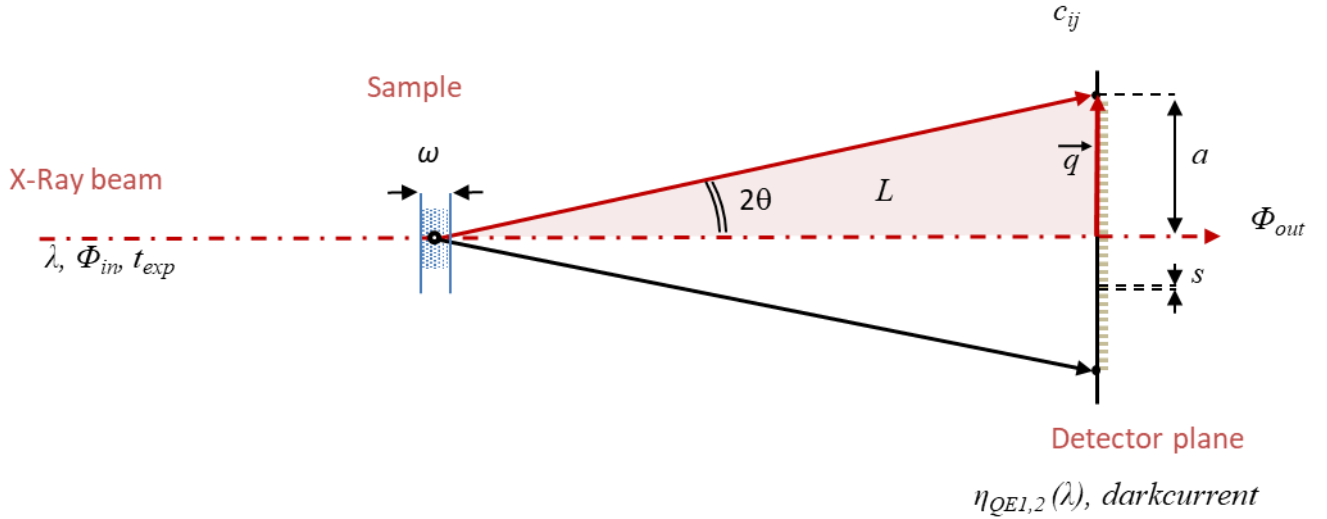


Figure 2 : Schematic diagram of the SAXS

From a geometrical perspective the modulus of momentum transfer  $q$  can be calculated using:

$$q = \frac{4\pi}{\lambda} \sin \theta \quad (1), \quad \text{with} \quad 2\theta = \arctan\left(\frac{a}{L}\right) \quad (2)$$

$2\theta$  being the total scattering angle of the scattered photons,  $L$  being the distance between the sample and the detector plane, and  $a$  being the distance between the point of normal incidence of the incident beam and detector. The SAXS detector counts single photons per pixel  $(i,j)$  such that the photon counts  $C_{i,j}$  need to be normalized by the thickness of the sample  $\omega$ , the experimental time  $t_{exp}$ , the solid angle of the scattered beam  $\Delta\Omega$ , the quantum efficiency of the pixel detector  $\eta_{QE2}$ , the quantum efficiency  $\eta_{QE1}$  of the detector measuring the outgoing photon flux  $\phi_{out}$  to receive the total scattering cross-section  $\frac{d\Sigma}{d\Omega}$  per volume  $V$  of suspended nanoparticles :

$$\frac{d\Sigma}{d\Omega}(q) = \frac{\eta_{QE1}}{\eta_{QE2}} \cdot \frac{C_{ij} - \text{darkcurrent}}{\phi_{out}} \cdot \frac{1}{\Delta\Omega \cdot t_{exp} \cdot \omega} \quad (3)$$

The solid angle of the scattered beam can be calculated using:  $\Delta\Omega = \left(\frac{s}{L}\right)^2$  (4)

and the transmission  $T = \frac{\phi_{out}}{\phi_{in}}$  (5)

Depending on the instrument type (synchrotron or laboratory setup) and detector capabilities, it is possible to measure the primary beam directly, then measuring  $\phi_{out}$  and  $C_{ij}$  on the same detector, avoiding to take the quantum efficiency  $\frac{\eta_{QE1}}{\eta_{QE2}}$  into account.

### 3.2 Scattering intensity of the sample

The scattering intensity  $I_0(q)$  of a single spherical particle can be analytically solved as<sup>a</sup>:

$$I_0(q) = V^2 \cdot (\Delta\rho)^2 \cdot \left[ 3 \cdot \frac{\sin(qr) - qr \cos(qr)}{(qr)^3} \right]^2 \quad (6)$$

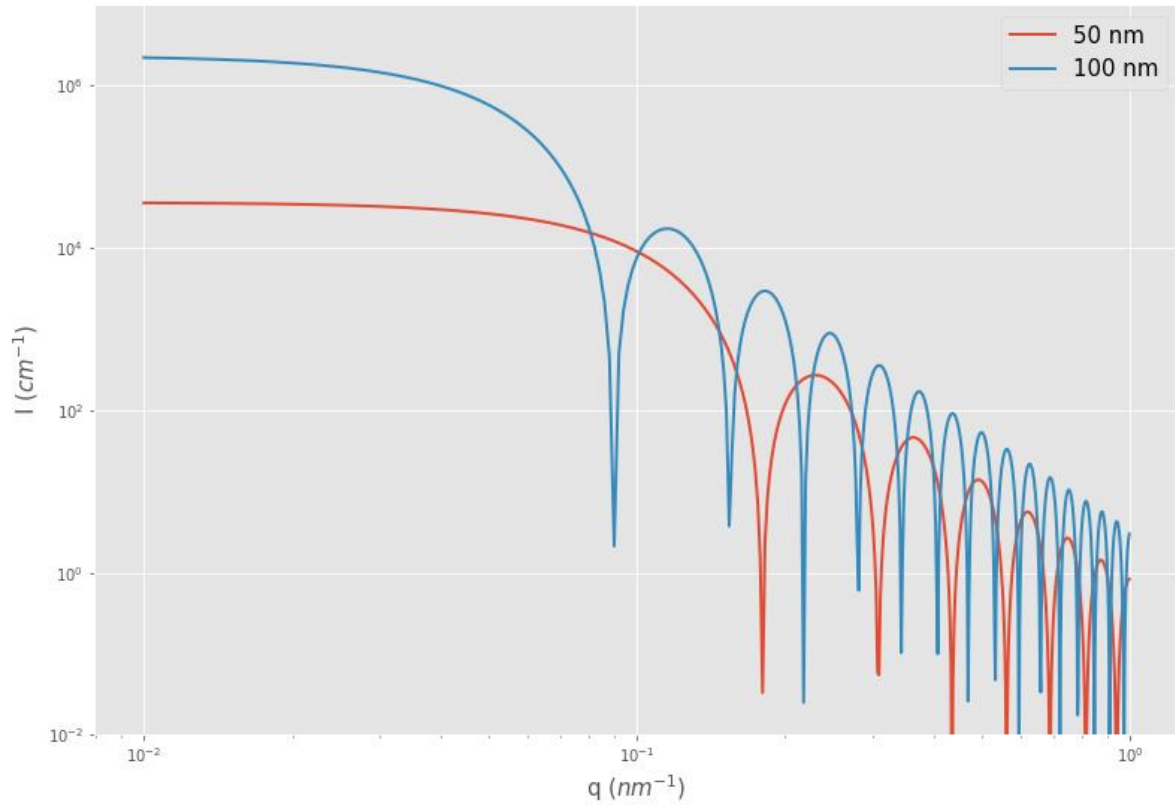


Figure 3 : Theoretical SAXS diagram of spherical particles suspension (red: 50 nm and blue: 100 nm size)

Where:  $V$  is the volume of the particle,  $V = \frac{4}{3} \cdot \pi \cdot r^3$  (7)

$r$  the radius of the sphere, and  $\Delta\rho = (SLD_{solvent} - SLD_{sphere})$  (8)

$SLD_{solvent}$  and  $SLD_{sphere}$  are the Scattering Length Densities (SLD in  $\text{cm}^{-2}$ ) of the nanoparticle sphere and the solvent respectively, and can be calculated:

$$SLD = \frac{\text{mass density } (\rho) \cdot \text{atomic number} \cdot \text{Avogadro constant}}{\text{atomic mass of the element}} \quad (9)$$

The scattering intensity  $I(q)$  of a number  $n$  of spherical particles, equals of the sum of the intensities  $I_0$  of same size single particles:  $I(q) = n \cdot I_0$  (10)

$n$  is the number concentration of spheres (number /  $\text{cm}^{-3}$ ).

Then, if  $d\Sigma/d\Omega$  (usually scaling in  $1/\text{cm}$  or  $1/\text{m}$ ) is known, **the number concentration  $n$  of the suspended particle ensemble can be determined.**

## 4. Uncertainty estimations for the concentration of spherical nanoparticles

Uncertainty estimations for the concentration of spherical particles depends on the contributions related to  $q$  and those related to intensity measurements (scattering cross-section). The estimations here are in-part based on published works from **PTB**<sup>c</sup> and **BAM**<sup>b</sup>:

All SAXS experimental contributions are:

CONTRIBUTION	DESCRIPTION	UNIT
$C_{ij}$	Measured intensities at pixel $i,j$	photons
$\Lambda$	Main wavelength	m
$\Phi_{IN}$	Incident flux	photons
$\Phi_{OUT}$	Transmitted flux	photons
$T_{EXP}$	Experiment time	s
$\Omega$	Sample Thickness	mm
$L$	Sample to detector distance	mm
$A$	Distance from beam center to $q$	mm
$S$	Pixel size	mm
$H_{QE1}$	Detector efficiency for $\phi_{out}$	
$H_{QE2}$	Detector efficiency for $C_{ij}$	
<b>DARKCURRENT</b>	Detector dark current	photons/pixel
$T$	Transmission	

### 4.1 Uncertainties in $q$

Uncertainties in  $q$  can be divided into two groups: those whose distributions are defined and can be taken into account in a data analysis procedure (sample thickness  $\omega$ , the beam profile, wavelength  $\lambda$ , as well as the detection location within the detecting volume), and true sources of uncertainty. The latter uncertainties include uncertainties in sample-to-detector distance ( $L$ ) determination, uncertainties in pixel size ( $s$ ), and can include uncertainties in the wavelength if they cannot be measured.

The uncertainties in sample-to-detector distance can be determined within 1 mm over the distance. In order to obtain  $q$  range adapted to nanoparticle analysis (between 10 nm to 100 nm), the sample-to-detector distances (depending on the chosen configuration and wavelength) is between ca. 500 mm to 2000 mm.

CONTRIBUTION	LABORATORY EST. STANDARD DEV <sup>B</sup>	SYNCHROTRON EST. STANDARD DEV <sup>C</sup>
$\Lambda$ , WAVELENGTH	0.25 %	0.01 %
$L$ , SAMPLE-TO-DETECTOR DISTANCE	0.5 %	0.2 %
$S$ , PIXEL SIZE	0.2 %	0.2 %
<b>TOTAL</b>	0.6 %	0.3 %

**The uncertainty in  $q$  can be less than 1% for laboratory instruments and less than 0.5% for Synchrotron instruments.**

However, in this uncertainty contribution only a common scaling factor in  $q$  is reported. Due to the influence of the beam centering, the uncertainty of  $q$  is dependent on the distance from the beam center. As an example, the figure below shows this dependency for a few typical configurations at the **BAM** MAUS instrument.

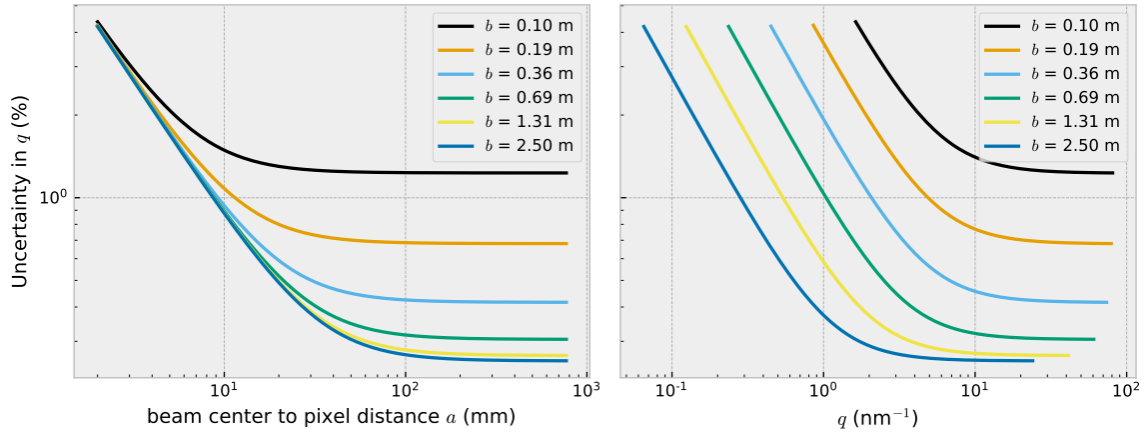


Figure 4 : The relative uncertainty in  $q$ ,  $\frac{\delta q}{q}$ , as a function of  $\alpha$  (left) and  $q$  (right) for several sample-to-sample detector distances  $b$ .

#### 4.2 Traceability and uncertainties in scattering cross-section $I$

The first contribution in scattering cross section intensities comes from the experimental measurements of the intensities according to equation (3).

CONTRIBUTION	LABORATORY EST. STANDARD DEV <sup>B</sup>	SYNCHROTRON EST. STANDARD DEV <sup>C</sup>
Incident flux $\varphi_{in}$	0.071 %	1 %
Transmission	0.23 %	1 %
Solid angle $\Delta\Omega$	1 %	0.2 %
Sample thickness $\omega$	1 %	3 %
<b>TOTAL</b>	<b>1.4 %</b>	<b>3.3 %</b>

**The biggest uncertainties are derived from the determination of the sample thickness  $\omega$ .**

For instance, the **PTB** uses rectangular capillaries consisting of borosilicate glass with a homogeneous thickness  $\omega$  along its vertical axis. The uncertainty is larger for this setup with individual capillaries compared to a setup with a flow-through capillary. **CEA** and **BAM** use cylindrical glass or Kapton capillaries. Moreover,  $\omega$  of each single capillary can be evaluated by measuring the transmission of the empty capillary as well as the transmission of the same capillary filled with a fluid of known mass absorption coefficient (e.g. water).

The second type of contributions comes from the nanoparticle suspension themselves. We can extract the main parameters from equation (6). The radius of the particle  $r$  can be estimated from the signal oscillations in  $q$  with a very high accuracy.  $\rho$  strictly depends on the density of the elements. The atomic composition used for the calculation of the Scattering Length Density is assumed to be known to a high degree of accuracy, though literature values show a large span of range.

CONTRIBUTION	DESCRIPTION	UNIT	EST. STANDARD DEV CONTRIBUTION TO N	DETERMINED BY:
$r$	Radius of the particle	Å	6 %	Q uncertainties
$\rho$	Density of particles	g/cm <sup>3</sup>	5 %	Known atomic composition <sup>b</sup>
<b>TOTAL</b>			7.8 %	

The total uncertainty contributions in intensity are in the range of 8% to 11% depending the sample thickness determination protocol and the density determination of the nanoparticles.

## 5. Conclusions and Outlook

In this report, we show that contribution comes primary from the uncertainty in  $q$  (less than 1%) and predominantly from the determination of the sample thickness and density (between 8% and 11%). For this we have partly compiled previous works on the estimation of uncertainties for the determination of nanoparticle concentration measurements with Small-Angle X-rays Scattering.

Different approaches can be taken to decrease such uncertainties: sample holder with very well-known thickness (flow through cells, extruded material capillaries, etc.). But previous EU projects<sup>d</sup> on metrological measurements shown that the density of materials can be hard to measure.

We didn't take into account the numerical and software contribution for the intensity estimation. For example, depending on the instrument, beam desmearing should be apply on the software for improving diameter size, and then reduce concentration uncertainties.

Different type of algorithms can also improve the model fitting on the experimental data.

## References

<sup>a</sup> ISO 17867:2015, « Particle size analysis — Small-angle X-ray scattering », s. d.

<sup>b</sup> Glen J. Smales et Brian R. Pauw, « The MOUSE Project: A Meticulous Approach for Obtaining Traceable, Wide-Range X-Ray Scattering Information », *Journal of Instrumentation* 16, n° 06 (1 juin 2021): P06034, <https://doi.org/10.1088/1748-0221/16/06/P06034>.

<sup>c</sup> Alexander Schavkan et al., « Number Concentration of Gold Nanoparticles in Suspension: SAXS and SpiCPMS as Traceable Methods Compared to Laboratory Methods », *Nanomaterials* 9, n° 4 (1 avril 2019): 502, <https://doi.org/10.3390/nano9040502>.

<sup>d</sup> InNanopart, « Metrology for innovative nanoparticles », s. d.

<sup>e</sup> Valérie Geertsen et al., « Contribution to Accurate Spherical Gold Nanoparticle Size Determination by Single-Particle Inductively Coupled Mass Spectrometry: A Comparison with Small-Angle X-Ray Scattering », *Analytical Chemistry* 90, n° 16 (21 août 2018): 9742-50, <https://doi.org/10.1021/acs.analchem.8b01167>.

<sup>f</sup> Christian Gollwitzer et al., « A Comparison of Techniques for Size Measurement of Nanoparticles in Cell Culture Medium », *Anal. Methods* 8, n° 26 (2016): 5272-82, <https://doi.org/10.1039/C6AY00419A>.

<sup>g</sup> Olivier Taché et al., « MOMAC: a SAXS/WAXS laboratory instrument dedicated to nanomaterials », *Journal of Applied Crystallography* 49, n° 5 (1 octobre 2016): 1624-31, <https://doi.org/10.1107/S1600576716012127>.