

# Standard Operating Procedures for Small-Angle X-Ray Scattering (SAXS) experiment

*Camille GUIOT and Olivier SPALLA, CEA, France*

*camille.guiot@cea.fr – olivier.spalla@cea.fr*

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This document describes the general procedure applied at CEA LIONS to perform Small Angle X-ray Scattering measurements and to treat the data. This procedure was applied in the framework of NANOGENOTOX to characterize TiO<sub>2</sub> and SiO<sub>2</sub> manufactured nanomaterials as raw powders and in aqueous suspensions.

## I. Description of the method and data collection

Here is a brief presentation of SAXS technique, for more details on this technique, cf references at the end of this document.

Small-Angle X-ray Scattering is a technique based on the interaction between X-rays and electrons to probe the structure of materials. The processed data is the number of X-ray scattered by a sample as a function of angular position of a detector (Figure 1).

The experimental set up (X-ray source, optical elements, detectors, etc) as well as the procedure for absolute scaling of data have been thoroughly described by Zemb and by Né (cf *Zemb 2003* and *Né 2000*).

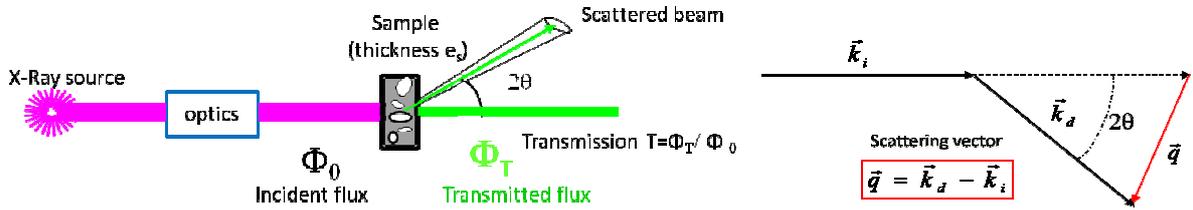


Figure 1: Schematic set up for SAXS and physical quantities

2D raw data images are converted into diffractograms displaying the **scattered intensity  $I$**  as a **function of scattering vector  $q$**  defined by:

$$q = \frac{4\pi \sin\theta}{\lambda}$$

$\lambda$ : X-ray wavelength

The experimental scattering intensity is defined as the differential scattering cross-section per unit volume of sample and can be expressed as follows:

$$I(q) = \frac{1}{V} \frac{d\sigma}{d\Omega} = \frac{\eta_1 C_{ij}}{\eta_2 (\Phi_0 S T) dt} \frac{1}{\Delta\Omega} \frac{1}{e}$$

$\sigma$ : scattering cross-section

$V$ : volume of sample

$C_{ij}$ : number of counts detected on a pixel  $ij$  during  $dt$

$\eta_1$ : detector quantum efficiency when measuring the direct beam

$\eta_2$ : detector quantum efficiency for the count  $C_{ij}$

$(\Phi_0 S T)$ : flux (in detector unit counts/s) integrated over the whole beam transmitted by the sample

$T$ : transmission of the sample

$\Delta\Omega$ : solid angle covered by one pixel seen from the center of the sample ( $\Delta\Omega = p^2/D^2$  with  $p$  the pixel size and  $D$  the sample to detector distance).

The intensity is then expressed in **absolute scale** (in  $cm^{-1}$ ) to be independent of the experimental set up parameters (X-ray wavelength, experimental background, time of acquisition, sample thickness, etc).

General theorems of experimental physics have been developed to extract different properties of nanostructured material from the diffractograms, such as, shape of nanoparticles, surface area, interactions occurring, etc.  $I(q)$  curves can also be theoretically calculated from assumed nanostructures to fit the experimental curves.

In the simple case of binary samples, the scattering intensity is proportional to:

- the electronic contrast, more precisely the square of scattering length density difference between the two materials  $(\Delta\rho)^2$ ,
- the concentration of the scattering object (in volume fraction), in case of suspensions for example.

### *Ultra Small angle X-ray Scattering (USAXS)*

Ultra Small Angle X-ray Scattering (USAXS) measurements give access to X-ray scattering data for a range of smaller  $q$  and then complement the SAXS diffractograms. It requires a specific and very precise set-up, different from the one used for SAXS.

## **II. Materials and chemicals**

### *Apparatus*

The main set up components used for SAXS and USAXS experiments at CEA/LIONS are listed hereunder :

- X-ray generator : Rigaku generator RUH3000 with copper rotating anode ( $\lambda= 1.54 \text{ \AA}$ ), 3kW
- Home made optic pathways and sample holders (with two channel-cut Ge (111) crystals in Bense/Hart geometry for USAXS set up, cf *Lambard 1992*).
- Flux measurement for SAXS set up : pico amperemeter Keithley 615
- Flux measurement for USAXS set up : DonPhysik ionization chamber
- Detector for SAXS set up : 2D image plate detector MAR300
- Detector for USAXS set up : 1D high count rate CyberStar X200 associated to a scintillator/ photomultiplier detector.

All experiment parameters are monitored by computer thanks to a centralized control-command system based on TANGO, and interfaced by Python programming. 2D images are treated using the software *ImageJ* supplemented with some specific plugging developed at CEA/LIONS. This control-command system have been achieved by Olivier Taché and is detailed in reference *Taché 2006*.

## Calibration

A sample of 3 mm of Lupolen® (semi crystalline polymer) is used for the calibration of the intensity in absolute scale, the maximum intensity being adjusted to  $6 \text{ cm}^{-1}$ .

A sample of 1 mm of octadecanol is used for the calibration of the  $q$  range (calculation of sample-to-detector distance), the position of the first peak standing at  $0.1525 \text{ \AA}^{-1}$ .

Calibrations in intensity and in  $q$  range are performed before each series of measurements.

## III. Sample preparation

Almost any kind of material can be analyzed by SAXS, whether it comes as a powder, a colloidal suspension, a gel, or even self-supported hybrid materials, and as long as the sample prepared meets some requirements of transmission and scattering properties.

Indeed, depending on the X-ray absorption coefficient of the material and its scattering properties, the sample thickness have to be adjusted to get a transmission as close as possible to the target transmission of 0.3 (optimal absorption/transmission ratio).

The sample thickness  $e$  is directly linked to the transmission  $T$  by the following equation:

$$e = -\frac{1}{\mu} \ln(T)$$

$\mu$ : X-ray absorption coefficient of the material,

$T$ : transmission,  $T = \text{transmitted flux} / \text{incident flux of the direct beam}$

If not self-supported (liquids, powders or gel), the material to be analyzed is inserted in a cell, which can be made of glass (capillary), or X-ray transparent material such as Kapton® (polyimide). In any case, a measurement of the empty cell is performed and subtracted as a background for the sample measurement.

Some examples of cells used for SAXS measurements at CEA/LIONS are shown on Figure 2.

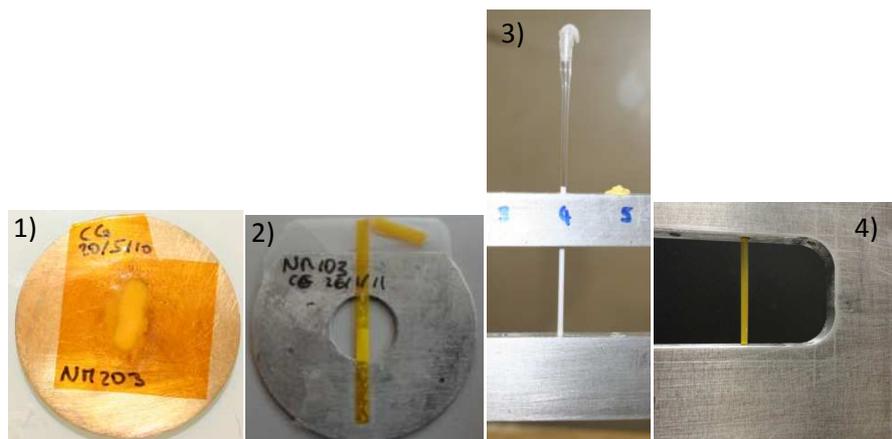


Figure 2 : Examples of different type of cells used for SAXS measurements, 1) double sticky kapton® cell for powders, 2) 1.5 mm flattened polyimide capillary for powders, 3) 1.5 mm glass capillary for powder or liquid samples, 4) 1.5 mm polyimide capillary for powder or liquid samples

### *Powder samples for NANOGENOTOX*

Nanomaterials in the form of powder are stored in glass vials of about 2 g sealed under argon. Once a vial has been opened to air for experiments, it is filled by N<sub>2</sub> after each use and the period of experiments should not exceed a few months. All experiments involving handling of powders are conducted under a fume hood with protective gloves, mask and glasses.

The coefficient of absorption depends on the material and on the energy. For the Cu K $\alpha$  emission (8 keV) that is used on our setup, the coefficient for TiO<sub>2</sub> is  $\mu_{\text{TiO}_2} = 470 \text{ cm}^{-1}$  and  $\mu_{\text{SiO}_2} = 77 \text{ cm}^{-1}$  for SiO<sub>2</sub>. The optimal sample thickness (equivalent thickness of dense material) to get a transmission of 0.3 is 25  $\mu\text{m}$  for TiO<sub>2</sub> and 150  $\mu\text{m}$  for SiO<sub>2</sub>.

In a first place, the TiO<sub>2</sub> powder samples were prepared between two sticky kapton® films pressed on a 0.4 mm brass cell (typical thickness of dense material around 30  $\mu\text{m}$ ). However, it was inferred that the presence of glue may affect the calculation of specific surface area of powders. Therefore, in a subsequent step, all the TiO<sub>2</sub> powder samples were measured in a flattened polyimide capillary, mounted on a circular sample holder (Figure 2, 2). The typical equivalent thickness of dense material obtained is 30  $\mu\text{m}$ .

Most of the SiO<sub>2</sub> powder samples were prepared in 1.5 mm glass capillaries leading to typical equivalent thickness of dense material from 100 to 200  $\mu\text{m}$ . However, NM203 powder is very sticky and was very difficult to insert into capillaries, so it was measured in a double sticky kapton® cell.

### *Aqueous suspensions for NANOGENOTOX*

All aqueous suspensions prepared for NANOGENOTOX were measured in 1.5 mm polyimide capillaries, leading to transmissions of about 0.25.

## IV. Measurement

In order to calculate the sample transmission, the flux of incident and transmitted beam are measured and averaged over 200 s before running the SAXS measurement.

The time of acquisition necessary for SAXS experiment depends on the sample properties. For SiO<sub>2</sub> and TiO<sub>2</sub> powders, two measurements were performed: one with a short time of 200 s or 150 s to get unsaturated data for small angles (low q), and one for a long time of 1800 s to get data in the high q region with low signal/noise ratio.

All aqueous suspensions were measured for 3600 s (typical concentration in oxide 3 g/L).

## V. Raw data treatment

### *SAXS data*

#### *Radial averaging of 2D image (ImageJ)*

2D images from the detector are converted into Intensity = f(scattering vector q) graphs thanks to the software ImageJ together with SAXS plugging. The process follows mainly these steps:

- Determination of the center coordinates (direct beam position)
- Application of a mask to remove pixels corresponding to the beam stop and around the photodiode
- Radial averaging of the intensity, knowing pixel size, sample-detector distance and wavelength (example of parameters in Figure 3), conversion of pixel position into scattering vector  $q$ , and creation of a .rgr file containing with  $I(q)$  data.

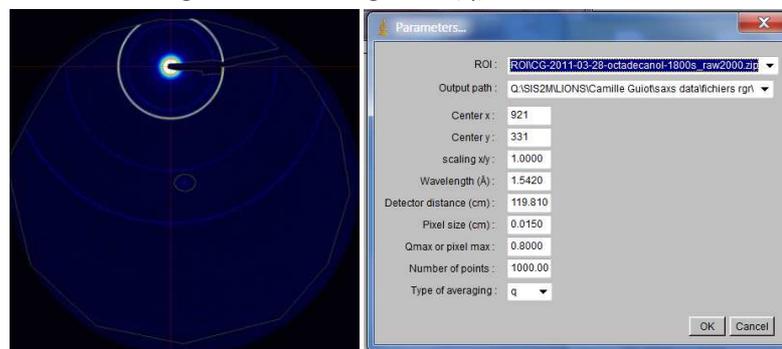


Figure 3 : Example of raw 2D image (octadecanol) and parameters used for radial averaging with ImageJ

#### *Absolute scaling of $I(q)$ (pySAXS)*

In order to scale the data to the absolute scale in  $\text{cm}^{-1}$ ,  $I(q)$  data generated by *ImageJ* as .rgr files are treated thanks to a homemade program called *pySAXS* and based on python programming.

The scaling involves a subtraction of the detector background and normalization by exposition time, sample transmission, sample thickness and K constant. The K constant is calibrated with Lupolen® sample and allows conversion of intensity in photon/s into absolute intensity in  $\text{cm}^{-1}$ . Example of parameters used for the scaling is shown on Figure 4.

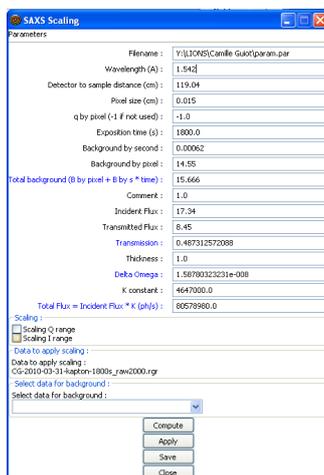


Figure 4 : Example of SAXS scaling parameter file from *PySAXS* software

The subtraction of the empty cell signal and the normalization by the sample thickness are then done in a subsequent.

## USAXS data

Raw USAXS data are generated as intensity vs angle data in .txt files. Data treatment is achieved using *PySAXS* and involves the following steps:

- Subtraction of the “rocking curve” (signal with empty cell) normalized by the intensities at  $0^\circ$  (transmission).
- Desmearing, taking into account the effective size of the “punctual” detector (cf *Lambard 1992*)
- Conversion of angle into q range
- Normalization by the sample thickness.

## VI. Data analysis

General theorems of X-ray scattering have been developed to analyze SAXS data. Here are presented some simple laws for **binary systems** (two phase samples), that may be of use in NANOGENOTOX framework.

### *Porod's Law*

In the high  $q$  range, sample diffractograms display an intensity decreases in a  $q^{-4}$  trend, called the “Porod region”. This region corresponds in the “real space” to the scale of the interfaces (for smooth interfaces).

Therefore, for a binary sample, the asymptotic limit of the so-called “Porod’s plateau”, when data are represented in  $Iq^4$ , is related to the total quantity of interface  $\Sigma$  (in  $m^2/m^3$ ) between the two phases, as follows:

$$\Sigma[m^{-1}] = \frac{\lim_{\text{plateau}}(I \cdot q^4)}{2\pi(\Delta\rho)^2}$$

Where  $\Delta\rho$  is the difference in scattering length density between the two phases.

For a binary sample of **known thickness**, the volume fraction of a material A  $\varphi_A$ , its specific surface area  $S_A/V_A$  (surface developed/ volume of A in the binary sample) and  $\Sigma$  are linked by the following relation:

$$\Sigma[m^{-1}] = \frac{S_A}{V_A} \varphi_A$$

For example, for a suspension of oxide in water, the determination of Porod plateau gives access to the concentration of the sample if the specific surface area of particles suspended is known (and vice versa).

### *Specific surface area determination from SAXS on powders*

To treat raw SAXS data and get absolute intensities, one needs to normalize the intensity by the thickness of the scattering material. However, in powder samples, the sample thickness is not clearly defined and cannot be precisely controlled since it depends on the powder compaction and the different scales of porosity. To elude this problem, a model system is used, in which we consider the effective thickness of material crossed by X-rays, called  $e_B$ , corresponding to an equivalent thickness

if all the material was arranged in a fully dense (no inner or outer porosity) and uniform layer (Figure 5).

The sample transmission is related to this equivalent thickness by the following equation:

$$e_B = -\frac{1}{\mu} \ln(T_{exp})$$

$\mu$  : material absorption coefficient for X-Ray -  $\mu_{TiO_2} = 470 \text{ cm}^{-1}$  -  $\mu_{SiO_2} = 77 \text{ cm}^{-1}$

$T_{exp}$  : **experimental transmission** (transmitted flux  $\Phi_T$ / incident flux  $\Phi_0$ ), i.e. transmission of the sample with regard to the transmission of the empty cell (kapton® alone, empty capillary, etc).

The intensity scaled by this thickness  $e_B$  is called  $I_1$ . The Porod's law can then be applied for  $I_1$  to access the specific surface area of the powder. Cf *Spalla 2003* for more details on the data analysis.

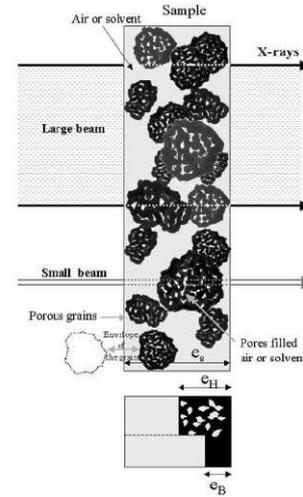


Figure 5: Schematic representation of a powder sample for SAXS measurement, and definitions of equivalent thicknesses  $e_H$  and  $e_B$ .

### Invariant theorem

When  $I(q)$  can be extrapolated to zero values of  $q$  (no interaction at a large scale, i.e. a flat signal for low  $q$ ) and infinite  $q$  (usually with the Porod law), the following invariant theorem can be applied:

$$Q = \int_0^{\infty} I_{Abs} q^2 dq = 2\pi^2 \varphi (1 - \varphi) (\Delta\rho)^2$$

This implies that the invariant  $Q$  is a constant for a defined composition, which gives access to the volume fraction  $\varphi$ , or to the evolution of interactions for a fixed composition.

### Guinier regime

For **diluted samples of monodispersed objects** (negligible position correlation between scattering objects, i.e. structure factor 1), the intensity in the low  $q$  region ( $qR_G \ll 1$ ) can be approximated to:

$$I(q) \approx A \left( 1 - \frac{(qR_G)^2}{3} + Bq^3 \right)$$

Which gives access to the **radius of gyration of the particles  $R_G$**  with the slope of  $\ln(I)=f(q^2)$ .

## *Data fits*

Assuming values of parameters such as volume fraction, size, shape and polydispersity of scattering objects for a model sample, it is possible to calculate theoretical curves of  $I(q)$ . Therefore, the adjustment of such parameters to fit theoretical curves with some experimental data allows for the modelisation of the sample properties.

## **VII. References**

*Zemb 2003* T. Zemb, O. Taché, F. Né, and O. Spalla; “A high-sensitivity pinhole camera for soft condensed matter”; JOURNAL OF APPLIED CRYSTALLOGRAPHY, 36, 800-805, **2003**.

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