Size Determination of Nanoparticles with Small-angle X-ray Scattering

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Small-angle X-ray scattering (SAXS) is an established method for the dimensional characterization of objects in the nanometer range [1]. It allows, for example, the size determination of micro- and nanoparticles with mean diameters in the range between a few nanometers and approx. 300 nm. Metrological traceability to the International System of Units (SI) has been achieved at PTB's laboratory at BESSY II in the past few years for sufficiently monodisperse nanoparticles; this is highly relevant to numerous applications in dimensional nanometrology. But dimensional investigations can also be carried out on nanoparticles with a broader size distribution. This article provides an overview of the method, of the experimental set-up used at PTB, and of the analysis of SAXS measurements; it shows examples of the traceable size determination of reference materials and the investigation of biological nano-objects.

In this measurement procedure, an almost parallel and monochromatic X-ray beam hits a sample which is present in a liquid, for example in the form of a suspension of nanoparticles, and is located within a thin-walled glass capillary tube. It is also possible to examine solid samples, for example to determine the pore size. In both cases, the X-rays are scattered forward due to discontinuities in the electron density, i.e. under small angles in relation to the direction of the radiation, and are then registered by an area detector (Figure 1). Hereby, the scattered radiation is less intensive than the transmitted beam by several orders of magnitude and it decreases further from the paraxial to the edge region, again by several orders of magnitude. The direct beam must therefore be shielded by means of a beamstop in order not to overload the detector. The scattering pattern is analyzed by comparison with mathematical models; contrary to microscopic techniques such as electron or scanning force microscopy, one measurement suffices to immediately obtain the mean size of a large number of particles which interests the most. Furthermore, sample preparation is not particularly tedious. SAXS is thus the ensemble method used at PTB when it comes to determining the size of nanoparticles [2].

SAXS measurements require intensive, monochromatic X-rays of low divergence. Synchrotron radiation is therefore best suited. At PTB's laboratory, the four-crystal monochromator (FCM) beamline is used; it covers the photon energy range from 1.75 keV to 10 keV [3, 4]. Up to 15 filled capillary tubes can be introduced simultaneously into the X-ray reflectometer [5, 6] which serves as the sample container. A large-area, spatially resolving detector is used to record the scattering pattern. To be able to measure across the full energy range of the beamline, a specially developed, vacuum-compatible hybrid pixel detector (Dectris PILATUS 1M) with a total surface of 179 mm × 169 mm and a pixel size of 172 µm is used [7]. The distance between the sample and the detector can be varied continuously between 2.2 m and 4.3 m using the SAXS facility of the *Helmholtz-Zentrum Berlin* (HZB) which is operated several times a year at the FCM beamline [8].

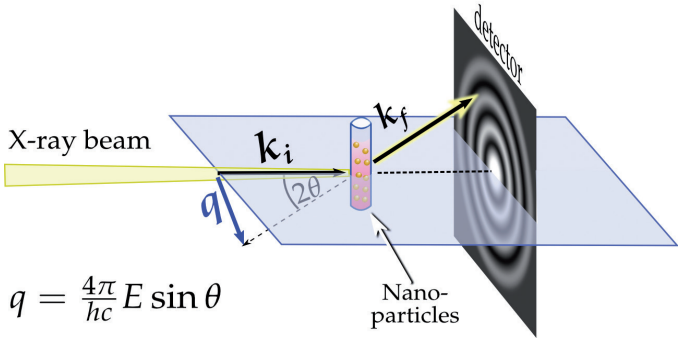

Figure 1:
Principle of SAXS measurements.

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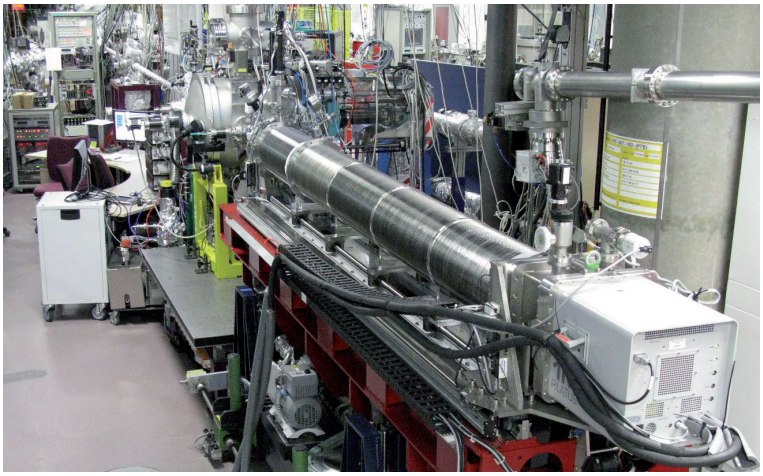

Figure 2:
SAXS instrument of the *Helmholtz-Zentrum Berlin* (HZB) at the FCM (four-crystal monochromator) beamline at PTB's laboratory at BESSY II. The directly connected vacuum-compatible PILATUS detector is visible at the bottom right.

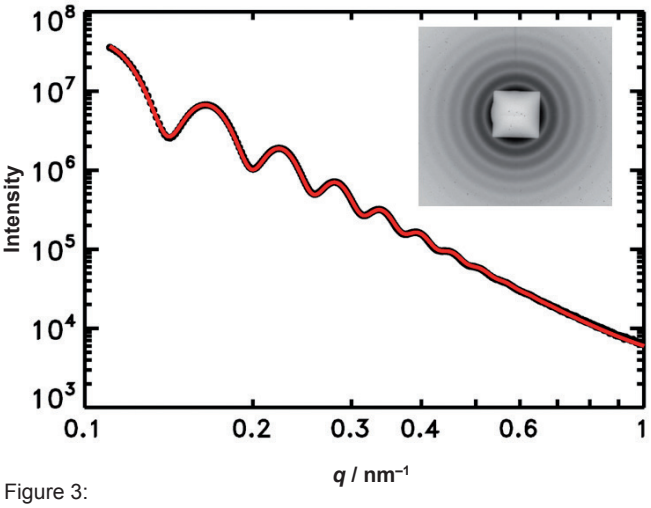

Figure 3:
Scattering curve for almost monodisperse PMMA particles with a nominal diameter of 108 nm. The fit yields a mean diameter of (109.0 ± 0.8) nm.

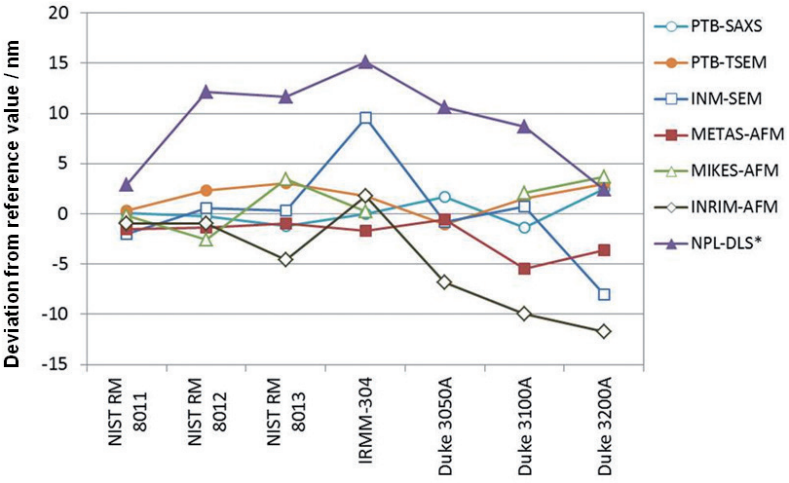

Figure 4:
Result of a European project on the traceability of the determination of the size of nanoparticles with 5 different measurement methods at 6 national metrology institutes. The particles in the 7 samples investigated had mean diameters from 10 nm to 200 nm.

To investigate nanostructured surfaces in reflection geometry at grazing incidence (GI)-SAXS (which is described in another article of this publication [9]), the whole facility can also be tilted by up to 3°. Also nanoparticles located on surfaces can be investigated using GISAXS [10].

In a normal SAXS geometry, the scattering pattern of monodisperse particles (i.e. particles with a narrow size distribution) consists, as shown in Figure 3, of concentric circles. By averaging, the radially symmetric scattering pattern can be reduced to one-dimensional scattering curves which then only depend on the scattering angle. For further computation, the amount q of the scattering vector is introduced; it is defined by the following relation:

$$q = \frac{4\pi}{\lambda}\sin\theta = \frac{4\pi E}{hc}\sin\theta \quad .$$

Hereby, λ is the wavelength and E is the energy of the X-rays, and θ is half the scattering angle. A scattering curve obtained in this way for monodisperse PMMA nanoparticles with a nominal diameter of 108 nm is shown in Figure 3, together with the original scattering pattern [8]. The square in the center is the shadow of the beamstop which blocks off the direct beam transmitted through the sample. In the calculation of the scattering curve, the transmission of the sample has been taken into account, and the scattering of a water-filled capillary tube has been subtracted.

The scattering curve can be fitted using an analytical model. Similar to the determination of the layer thickness by means of X-ray reflectometry [6], here too, the periodicity of the observed oscillations serves size determination, and here too, exact knowledge of the wavelength is exploited which, contrary to light scattering in the visible range, is considerably smaller than the objects under investigation. Whereas the period of the oscillations only depends on the mean particle diameter in the case of compact, spherical objects, besides the distribution width, also the parameters of the background enter into the amplitude, which results in higher uncertainties in this case. For monodisperse particles which can be described as homogeneous spheres, SAXS, however, meanwhile provides results that can be traced to the SI system for the mean diameter with very low uncertainties, as shown in Figure 4, where the results of a method comparison of European metrology institutes is depicted [11]. For the nanoparticles of a size in the range from 10 nm to 200 nm which were investigated using different methods, the values obtained with SAXS at PTB's laboratory exhibited the smallest deviation from the corresponding reference value. The determination

of the sample/detector distance, the pixel size of the detector and the adjustment of the model are the main contributions to the uncertainty. With suitable particles, relative uncertainties of 1 % can be achieved.

Figure 5 shows the more complex case of a bidisperse size distribution which is due to the mixing of two monodisperse fractions. For this mixture, the mean diameter and the distribution widths of the two components were determined in agreement with the results obtained separately with each of the two components [8].

Particularly interesting applications of small-angle X-ray scattering are encountered in the field of biological nano-objects and in nanoparticles in biological media. For particles made of materials with relatively low density (e.g. polystyrene), the adsorption of proteins can be detected by an increase in diameter. As shown in Figure 6, the protein shell becomes thicker when the concentration of, e.g., immunoglobulin G (IgG) increases in the surrounding solution [12]. In contrast, in the case of heavy particles, the influence of the shell is very small so that, as opposed to other procedures, the diameter of the actual particles can always be determined.

Biologically relevant nano-objects mostly exhibit a relatively wide diameter and shape distribution; equidistant circles are therefore seldom. But here too, there can be distinct structures, as shown in Figure 7 in the scattering curve for synthetic phospholipid vesicles which serve as a model system for biological membranes [13]. The size of the vesicles can be determined at small q values, whereas the thickness of the double layer is obtained from scattering curves at high q values.

Natural extracellular vesicles are present in all body fluids. Within the scope of a European metrology project, SAXS is used also here in combination with other technologies for size determination. Besides the large distribution width, also the low concentration of the vesicles and the existence of other particles in a similar size range are a challenge. With samples prepared with the greatest care, it was, however, possible to achieve first results. The scattering curve for erythrocyte vesicles shown in Figure 8 can be described by a vesicle distribution with a mean diameter of 125 nm and a full width at half maximum of the size distribution of 90 nm, as well as by a contribution of clearly smaller free proteins [14].

Also other biological issues such as, e.g., the influence of DNA and of heparin on the mechanical stability of blood clots were investigated at PTB's laboratory at BESSY II using SAXS [15]. For deeper investigations, also interesting is the so-called *anomalous small-angle X-ray scattering* (ASAXS), which exploits the change in the scattering intensity in the vicinity of an absorption

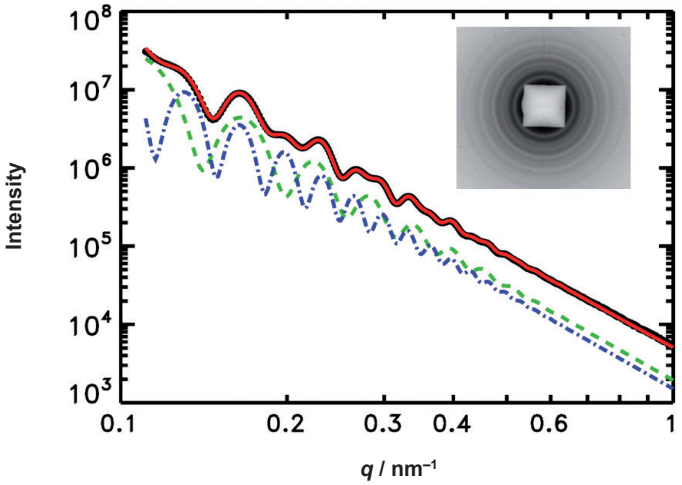

Figure 5:
Scattering curve for a mixture of PMMA particles with nominal diameters of 108 nm and 192 nm. The fit provides both size distributions with mean values of 109.2 nm and 188.2 nm.

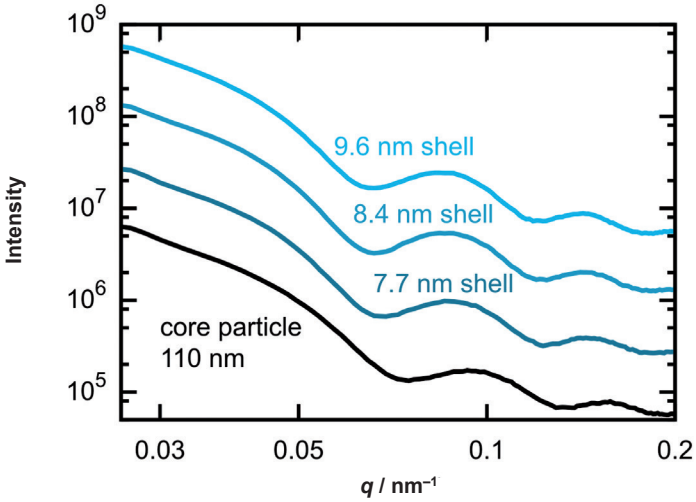

Figure 6:
Size increase of polystyrene nanoparticles by adsorption of proteins at increasing concentrations of immunoglobulin G (IgG).

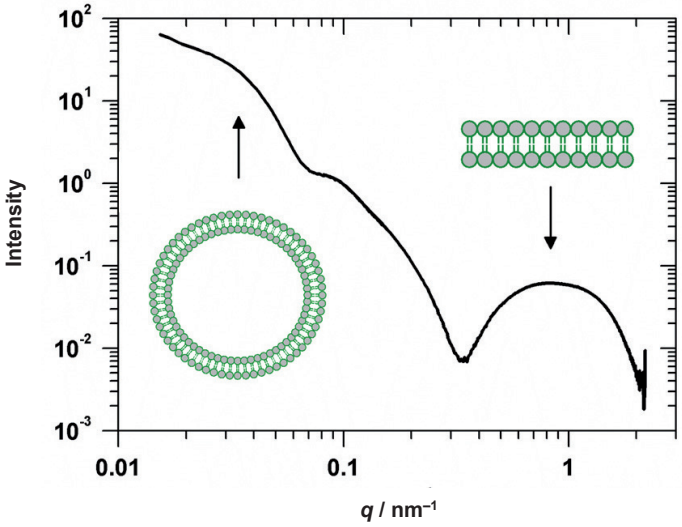

Figure 7:
Scattering curve for phospholipid vesicles from which a mean diameter of 75 nm can be determined at small q values and a thickness of the double layer of 4 nm at high q values [13].

edge. It allows statements, for example, on the distribution of chemical elements in core/shell systems. With the vacuum-compatible hybrid pixel detector at the FCM beamline, unlike most other SAXS facilities worldwide, also the adsorption edges of the biologically relevant elements calcium, potassium, chlorine, sulphur and phosphor are accessible. ASAXS investigations on the Ca edge have already been successfully completed [16].

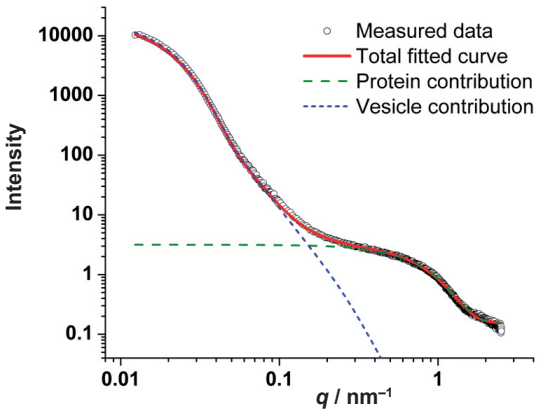

Figure 8:
Scattering curve for erythrocyte vesicles which can be described by a vesicle distribution with a mean diameter of 125 nm and a full width at half maximum of the size distribution of 90 nm as well as by a contribution of clearly smaller free proteins.

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