research papers

On the absolute calibration of bench-top small-angle X-ray scattering instruments: a comparison of different standard methods

Cécile A. Dreiss,[a]*‡ Kevin S. Jack[b] and Andrew P. Parker[c]

[a]School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK, [b]Centre for Nanotechnology and Biomaterials, School of Molecular and Microbial Sciences, The University of Queensland, QLD 4072, Australia, and [c]Bristol Colloid Centre, University of Bristol, University Gate, Park Row, Bristol BS1 5UB, UK. Correspondence e-mail: cecile.dreiss@kcl.ac.uk

Absolute calibration relates the measured (arbitrary) intensity to the differential scattering cross section of the sample, which contains all of the quantitative information specific to the material. The importance of absolute calibration in small-angle scattering experiments has long been recognized. This work details the absolute calibration procedure of a small-angle X-ray scattering instrument from Bruker AXS. The absolute calibration presented here was achieved by using a number of different types of primary and secondary standards. The samples were: a glassy carbon specimen, which had been independently calibrated from neutron radiation; a range of pure liquids, which can be used as primary standards as their differential scattering cross section is directly related to their isothermal compressibility; and a suspension of monodisperse silica particles for which the differential scattering cross section is obtained from Porod's law. Good agreement was obtained between the different standard samples, provided that care was taken to obtain significant signal averaging and all sources of background scattering were accounted for. The specimen best suited for routine calibration was the glassy carbon sample, due to its relatively intense scattering and stability over time; however, initial calibration from a primary source is necessary. Pure liquids can be used as primary calibration standards, but the measurements take significantly longer and are, therefore, less suited for frequent use.

1. Introduction

Absolute calibration relates the measured intensity to the differential scattering cross section of the sample, which contains the information specific to the material. Absolute calibration is necessary for the subsequent analysis and interpretation of the data. It is fundamental for obtaining parameters such as the molecular mass, the specific surface of the particles or the scattering length density. It is also a way of detecting the occurrence of multiple scattering or aggregation in the system. Additionally, absolute data allow the comparison of results obtained from different scattering techniques over different q ranges (Lindner, 2002; King, 1999).

The absolute intensity, which is the ratio of the scattering intensity to the primary intensity, can be obtained in two ways: a direct determination of the incident beam following its mechanical attenuation, or the use of standards of known differential scattering cross section. The general methods available for absolute scaling of scattering data have been described extensively (Lindner, 2002; Wignall & Bates, 1987;

Russell *et al.*, 1988; Zemb *et al.*, 2003). Moreover, absolute calibration for small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) is routinely performed at large-scale facilities, *e.g.* synchrotron and neutron facilities. However, the absolute calibration of laboratory-based 'bench-top' (SAXS) instruments appears to be less of a routine procedure, with results often reported in arbitrary units. This situation possibly arises from the perception that the procedures are both time consuming and more problematic on laboratory-based instruments for several reasons. These include the relatively weak scattering of readily available primary standards coupled with the lower sensitivity of the bench-top instrument, difficulties in obtaining stable pre-calibrated secondary standards, and the practical difficulties, *e.g.* removing and realigning of the beam-stop, when calibrating on a routine basis (Russell, 1983; Orthaber *et al.*, 2000).

Water has long been used as a secondary standard for the calibration of SANS data because of its strong incoherent background (Lindner, 2002; Wignall & Bates, 1987). However, it is not as widely employed in laboratory-based SAXS measurements due to its low scattering cross section, leading

‡ Presently at King's College London, Pharmacy Department, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK.

to rather poor accuracy of the data. It does, however, have advantages in that it is cheap, readily available and its coherent scattering depends on the isothermal compressibility only and can, therefore, be calculated. Its use as a calibration standard for SAXS has been reported for higher flux sources such as slit collimation systems (Dingenouts *et al.*, 1999; Orthaber *et al.*, 2000). Recently, Pedersen reported a procedure for the flux-optimization of a pinhole collimated instrument (a Bruker NanoStar), coupled with a higher flux rotating-anode source, which greatly enhanced the routine use of water as a primary standard (Pedersen, 2004).

Similarly, other pure liquids can be used as primary standards as their absolute scattering cross section depends only on the isothermal compressibility. The use of pure organic solvents has been described previously (Dingenouts *et al.*, 1999; Zemb *et al.*, 2003). Additionally, Zemb *et al.* have proposed the use of buffer solutions, which have larger cross sections, as alternative primary standards for SAXS calibration (Zemb *et al.*, 2003). The use of colloidal dispersions (both sols and solid dispersions) has also been detailed by Russell (1983) and such suspensions were found to be effective as primary standards provided that the interfacial region is well defined and hence the differential scattering cross section can be obtained from Porod's law. However, the long-term stability of colloidal suspensions is a cause for concern.

The uses and merits of amorphous carbon as a secondary standard have also been discussed in the literature (Russell *et al.*, 1988). These standards were shown to have a suitably high scattering cross section; however, the cross section needs to be determined experimentally and, in addition, it was noted that freshly made samples of amorphous carbon may not be stable with respect to time (Russell *et al.*, 1988).

In this report, we investigate and compare a range of standards for calibrating a standard 'bench-top' SAXS instrument in a single body of work. These include: water and other pure solvents as primary standards; glassy carbon as a secondary standard, following the measurement of its differential scattering cross sections by SANS; and a suspension of monodisperse silica particles of known size, for which the differential scattering cross section is obtained from Porod's law. Their suitability, in terms of accuracy, ease of use and stability over time, is then discussed, providing alternatives for different situations, such as availability of standards. In addition, as a result of the absolute calibration of our bench-top instrument, we were able to perform a detailed study of the interaction potentials in concentrated colloidal silica dispersions (Qiu *et al.*, 2006). This work further illustrates the importance of the determination of absolute intensity and demonstrates the quality of data that can be obtained on a calibrated bench-top instrument.

In the following section, we briefly review the notations and theoretical expressions used to derive the intensity in absolute units. We then present the calibration procedure carried out with pure liquids, glassy carbon and a silica sol as standard samples. In the last section, we compare and discuss the results obtained for the different standard samples and suggest an approach for rapidly calibrating after routine adjustments to

the instrument setup, *e.g.* camera length, radiation flux or collimation settings of a low-flux instrument. It should be noted that although the calibration factors determined here are specific to a given geometrical setup (collimation of the beam, detector distance, *etc.*), the methods described are applicable to different SAXS instruments and geometries, and the general conclusions are of direct relevance to the calibration of any type of 'bench-top' X-ray device.

2. Principles and theory

The derivation of the relevant expressions for absolute calibration can be found in other publications (such as Lindner, 2002, or King, 1999). In the following, we reiterate some important results and introduce the notations used below.

The intensity scattered from a sample as a function of the momentum transfer q is given by:

$$I_S(q) = \frac{N_S(q)}{t_S} = I_0(\lambda) A \, \Delta\Omega \, \eta(\lambda) T_S(\lambda) d_S \left(\frac{\partial\Sigma}{\partial\Omega}\right)_S (q) + BG_S,$$

(1)

with $q = 4\pi/\lambda \sin(\theta/2)$, where θ is the scattering angle. $I_S(q)$ is the quantity measured during an experiment and corresponds to the number of photons (N_S) of a given wavelength scattered through the angle θ that arrive on a small area of the detector per unit time (t_S). $I_0(\lambda)$ is the incident flux (in units of photons s^{-1} cm^{-2}), A is the area illuminated by the beam, $\Delta\Omega$ is the solid angle element defined by the size of a detector pixel, η is the detector efficiency, $T_S(\lambda)$ is the transmission of the sample, d_S is the thickness of the sample, and BG_S is the scattering background. Finally, $(\partial\Sigma/\partial\Omega)_S$ is the differential scattering cross section (in units cm^{-1}); it is the quantity obtained from the absolute calibration of the measured intensity.

The absolute calibration is achieved by the use of a standard of known differential cross section $(\partial\Sigma/\partial\Omega)_{st}$. Expressing the intensity scattered by the standard [following equation (1)] and dividing by the same expression obtained for the sample, we obtain

$$\left(\frac{\partial\Sigma}{\partial\Omega}\right)_S (q) = \left(\frac{\partial\Sigma}{\partial\Omega}\right)_{st} (q) \frac{[I_S(q) - BG_S]}{d_S T_{S+CAP}} \frac{d_{st} T_{st+CAP}}{[I_{st}(q) - BG_{st}]},$$

(2)

where the subscript CAP refers to the capillary in which the sample and/or standard may be contained. In this equation, all the terms relating to the geometry of the instrument in equation (1) have cancelled out. Expression (2) relates the differential cross section of the sample to the differential cross section of the standard (which is known) to quantities measured during the scattering experiment: namely, the intensity and the transmission.

The measurement of the scattering from various standards and the subsequent data treatment leading to absolute calibration is presented in the following sections. From each measurement, a 'calibration factor', CF, is derived:

$$CF = \left(\frac{\partial\Sigma}{\partial\Omega}\right)_{st} (q) \frac{d_{st} T_{st+CAP}}{[I_{st}(q) - BG_{st}]}.$$

(3)

Table 1
Results obtained from the measurement of all solvents as calibration standards for the transmission, the number of counts per second, the absolute cross section (obtained from the isothermal compressibility) and the resulting calibration factor, CF.

	Water	Toluene	Ethanol	Ethylene glycol	Pentane	Cyclohexane
Transmission	0.42	0.76	0.66	0.47	0.82	0.69
Counts s^{-1}	7.2	11.6	11.3	8.2	13.3	12.0
Absolute cross section (cm^{-1})	1.65×10^{-2}	2.29×10^{-2}	2.58×10^{-2}	1.54×10^{-2}	3.37×10^{-2}	2.56×10^{-2}
Calibration factor	21.2	19.6	19.8	22.4	22.0	20.0

The intensity in absolute units is then obtained by multiplying the measured intensity $I_S(q)$ by CF (following correction by time, sample thickness and transmission). The results for the different standards studied in this work will be discussed based on a comparison of the values of CF obtained.

3. Experimental

The instrument on which the calibration was performed in this work is the original compact version of the Bruker AXS NanoStar small-angle X-ray scattering instrument. The instrument uses Cu $K\alpha$ radiation (1.54 Å) produced in a sealed anode tube typically operating at a current of 35 mA and a potential difference of 40 kV. The X-ray beam is focused and monochromated using cross-coupled Göbel mirrors (Schuster & Göbel, 1995, 1996). Parasitic scattering from previous collimation elements is removed by a 300 µm pinhole placed prior to the sample chamber. Liquid samples were placed in a sealed quartz capillary, whilst the solid glassy carbon was simply fixed into the path of the beam. The sample chamber and beam path is evacuated to around 1 Pa and all the measurements were performed at 293 K.

The scattered X-rays are detected on a two-dimensional multiwire area detector and can be converted to one-dimensional scattering as a function of momentum transfer (*e.g.* by radial averaging) using standard reduction techniques (King, 1999). The transmission of X-rays through the sample is determined by an indirect method, in which a strongly scattering material of known X-ray transmission (*i.e.* glassy carbon) is used to diverge a proportion of the transmitted (direct) beam onto the detector. The integrated counts per unit time over the whole detector can then be related to the relative intensity of the direct beam and hence the transmission of the sample.

The water used in this work was milli-Q water. The solvents used as calibration standards were purchased from Aldrich with >99% purity. A colloidal suspension of silica particles of 25 nm diameter was kindly supplied by Clariant, with a solid content of 30.0% (*w/w*) at a pH of 9. The suspension was diluted to 3.0% (*w/w*) solid content with milli-Q water. The amorphous (glassy) carbon sample used as a secondary standard was purchased from Goodfellow.

4. Calibration procedures

4.1. Use of pure liquids as primary calibration standards

For pure liquids, the scattering can be obtained from classical fluctuation theory (Guinier & Fournet, 1955). With an extrapolation to momentum transfer $q = 0$, the differential scattering cross section (in cm^{-1}) is equal to

$$\left(\frac{\partial\Sigma}{\partial\Omega}\right)_{st}(0) = \rho n_e^2 b_e^2 (\rho kT)\chi_T \tag{4}$$

where ρ is the concentration of scattering objects, n_e the number of electrons per object and b_e the Thomson factor ($b_e = 0.2818 \times 10^{-12}$ cm). For water at 293 K and 10^5 Pa, $\chi_T = 4.591 \times 10^{-10}$ Pa^{-1} (CRC, 1983), giving

$$\left(\frac{\partial\Sigma}{\partial\Omega}\right)_{H_2O}(0) = 1.65 \times 10^{-2} \text{ cm}^{-1} \tag{5}$$

The absolute cross sections calculated for water and the other pure solvents studied here are listed in Table 1.

As an example of the procedure used to determine the calibration factor from a pure solvent, the scattering from water is described here. The scattering and transmission of the empty capillary (CAP) and that of the same capillary filled with water (H_2O + CAP) are measured, along with the detector dark current (BG). The scattering of water alone is then obtained by (see also King, 1999)
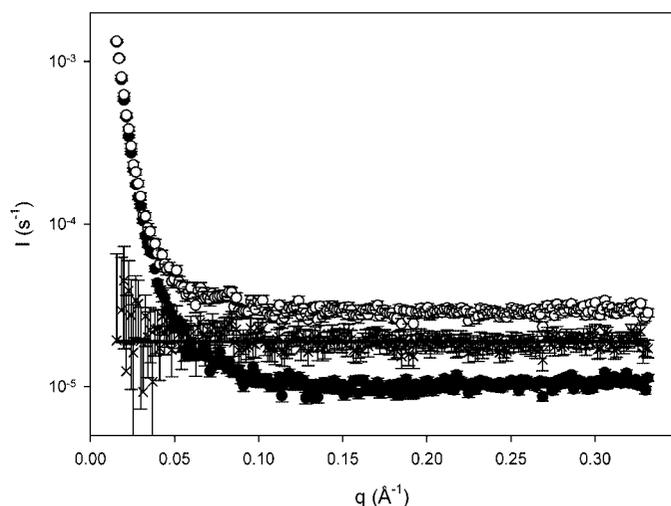
$$I_{H_2O}(q) = \left[I_{H_2O+CAP}(q) - BG\right] - T_{H_2O}\left[I_{CAP}(q) - BG\right]. \tag{6}$$

To obtain reasonable statistics using the experimental setup described above, it was necessary to make each measurement for *ca* 18 h (*ca* 5×10^5 integrated counts for water) or greater, making the total experimental time for a single calibration point of the order of >50 h. Additionally, it is noted that the dark current was found to be significant when compared with the scattering of the pure solvents and could not be neglected in the determinations.

The intensity profiles for water, with and without the scattering of the capillary, are shown in Fig. 1, where the 'dark current' has been subtracted from all samples. As expected, the scattering obtained is flat over the measured q range. Neglecting the points at very low q (which are not reliable due to some spreading of the beam around the beam-stop), the average value of the measured intensity (corresponding to the specific experimental conditions and instrument settings used) is determined by a zero-order least-squares analysis of the data points and gives

$$I_{H_2O} = 1.89 \times 10^{-5} \text{ s}^{-1}. \tag{7}$$

The standard deviation on the determination of this average value is 4%. With the measured transmission of water in the capillary being $T_{H_2O+CAP} = 0.266$, the calibration factor obtained from water is then given by

Figure 1
X-ray scattering of the empty capillary (filled circles), of the capillary filled with water (open circles), and water alone (crosses), after subtraction of the empty cell. The scattering from the empty capillary is multiplied by the transmission of water T_{H_2O}. Each sample was measured for two series of 18 h. The dark current was subtracted from all measurements. The continuous line is the fit to the flat background.
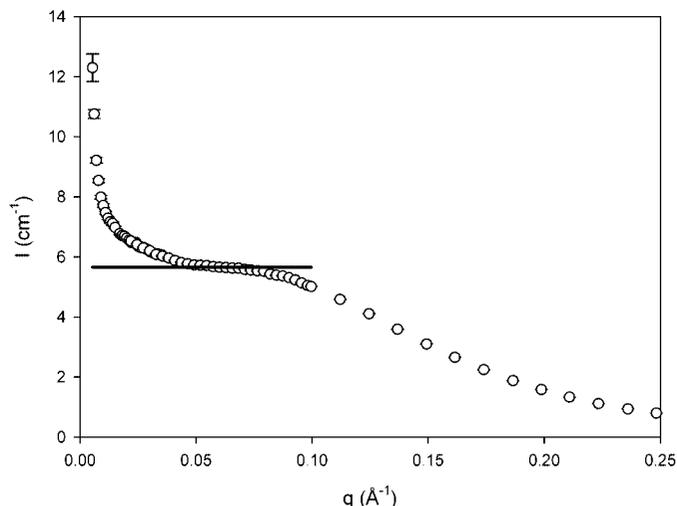
$$\mathrm{CF} = \frac{1.65 \times 10^{-2}}{1.89 \times 10^{-5}}\, 0.266\, d_{H_2O}. \tag{8}$$

The mean thickness of the curved water standard sample d_{H_2O} can be determined by a number of techniques, including determination from the sample transmission and volumetric or geometric measurements, all of which provide the same result within an error of 2%. Substituting the value $d_{H_2O} = 0.091$ cm into equation (8), we obtain for the calibration factor

$$\mathrm{CF}_{H_2O} = 21.2\ \text{s}. \tag{9}$$

That is, multiplication by CF of a measured scattering profile (background, transmission and sample-thickness corrected) collected with this experimental setup provides data with absolute intensity values.

Similarly, a number of pure organic solvents can be used as calibration standards as their absolute scattering cross section is obtained directly from their isothermal compressibility (CRC, 1983). To test the accuracy of the method, we have extended the measurements to a range of readily available organic solvents for which the absolute scattering cross section can be calculated: toluene, ethanol, ethylene glycol, pentane and cyclohexane. The results are presented in Table 1. There is good agreement between the calibration factors obtained from the different solvents. The average value is 20.8, with an error estimate of ±5.2% (*i.e.* twice the standard deviation, or 95% confidence interval). The variation observed between these values is within the combined uncertainty of the various sources of errors associated with this procedure. These are: the measurement of two transmissions, that of the sample and the empty cell, for which a standard deviation (σ) of 3% was obtained from a series of reproducibility measurements; the evaluation of the flat background ($\sigma = 4\%$); the value of the isothermal compressibility used, which varies with tempera-

ture ($\sigma = 1\%$); and the determination of the average thickness of the sample ($\sigma = 1\%$). The estimate of error in the CF determined for a single sample, obtained by adding the contributions from these various sources, is *ca* ±6%.

4.2. Use of glassy carbon as a secondary calibration standard

The glassy carbon specimen, of uniform 1 mm thickness, was measured by small-angle neutron scattering on D11, at the Institut Laue-Langevin (Grenoble, France). The intensity in absolute units was obtained as a function of momentum transfer q using the standard procedures (Ghosh *et al.*, 2000). The scattering is shown in Fig. 2. In order to determine the absolute differential scattering cross section for $q = 0$, a Debye–Bueche plot of $(\mathrm{d}\Sigma/\mathrm{d}\Omega)^{1/2}(q)$ *versus* q^2 is often used (Russell *et al.*, 1988). However, it is difficult to establish the linear region of the curve with certainty and hence a non-negligible error is obtained on the value of the intercept with the axis, which relates to the absolute cross section. Therefore, we chose to evaluate the neutron scattering cross section of the glassy carbon sample in one specific point situated in the low-q plateau (Fig. 2). The absolute intensity was determined for $q = 0.06$ Å^{-1}:

$$\left(\frac{\partial\Sigma}{\partial\Omega}\right)_{\mathrm{GC\_SANS}}(q = 0.06) = 5.66\ \mathrm{cm}^{-1}. \tag{10}$$

The corresponding X-ray scattering cross section is obtained by multiplying by the ratio of the scattering length densities (using tabulated values), $\Delta\rho_{\mathrm{SAXS}}^2/\Delta\rho_{\mathrm{SANS}}^2$, where it is assumed for the calculation that the sample consists of pure carbon:

$$\left(\frac{\partial\Sigma}{\partial\Omega}\right)_{\mathrm{GC\_SAXS}} = \left(\frac{\partial\Sigma}{\partial\Omega}\right)_{\mathrm{GC\_SANS}}\left(\frac{2.818 \times 6}{6.646}\right)^2 = 36.63\ \mathrm{cm}^{-1}. \tag{11}$$

The scattering from the glassy carbon was measured for 1.3 h (*ca* 10^7 integrated counts) and is shown in Fig. 3. Measurements of the background (1 Pa air) and the dark current were
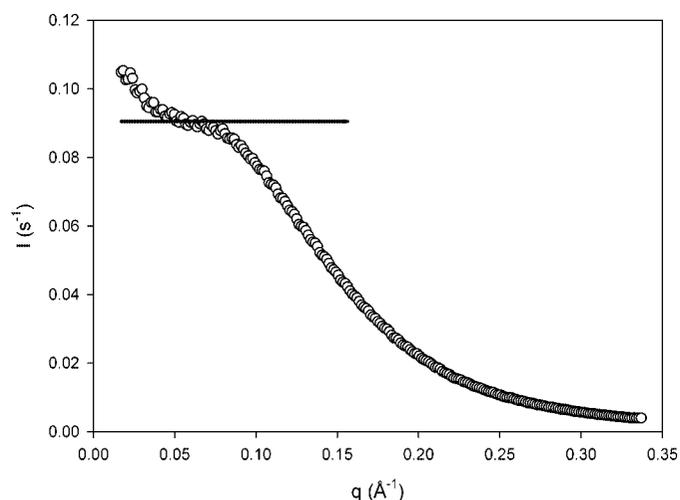

Figure 2
Small-angle neutron scattering from the standard sample of glassy carbon in units of cm^{-1}, measured on D11 (ILL, France).

Figure 3
Scattering from the glassy carbon standard sample measured for 1.3 h on the NanoStar SAXS instrument.
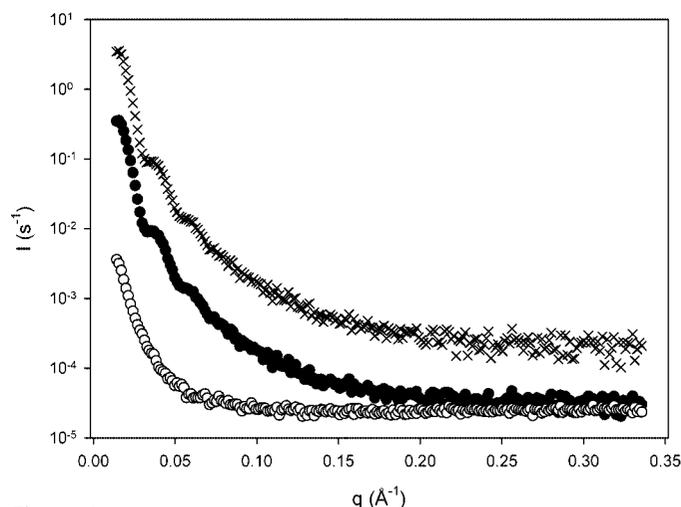

Figure 4
X-ray scattering of a 3% (w/w) suspension of silica contained in a quartz capillary (filled circles), of the empty capillary (open circles), and of the suspension of silica after subtraction of the empty cell (crosses) (scaled by a factor of 10, for clarity). The silica suspension was measured for 1 h, the empty capillary for 18 h and the dark current was subtracted from all samples. The error bars are smaller than the symbols and are not shown here.

also measured for a similar time and could be subtracted from the sample scattering as described above. However, these subtractions do not significantly influence the determination of the calibration factor for this sample and could be neglected. Several transmission runs were performed and averaged. A value of $T_{GC} = 0.527$ was obtained with a standard deviation of 0.6% on the measurements.

From Fig. 4, the intensity scattered at $q = 0.06$ Å^{-1} was measured as $I_{GC}(q = 0.06) = 0.090$ s^{-1} and the thickness of the standard sample of glassy carbon measured using a micrometer is $d_{GC} = 0.099$ cm. The calibration factor obtained with the glassy carbon standard is, therefore

$$CF_{GC} = \left(\frac{\partial \Sigma}{\partial \Omega}\right)_{GC}(q) \frac{d_{GC} T_{GC}}{I_{GC}(q)} = 21.1 \text{ s.} \tag{12}$$

In other studies, glassy carbon samples have been reported to evolve with time (Russell *et al.*, 1988), by absorbing or desorbing impurities, therefore altering significantly the value of $(\partial \Sigma / \partial \Omega)_{GC}$ and making them less attractive as a secondary standard. The neutron scattering profile of the glassy carbon sample used in this study was measured three times, on two different instruments (D11, at ILL, and LOQ at ISIS, Didcot, UK) and with a maximum time gap of five years between the measurements. The overall standard deviation obtained on the three measurements is 2%, which is within the experimental error obtained on absolute calibration. The glassy carbon specimen used in this work is, therefore, considered to be stable with time.

4.3. Use of a silica suspension as a calibration standard

Silica sols or silica particles in elastomeric matrices have been used as calibration standards to determine the incident-beam intensity (Russell, 1983; Russell *et al.*, 1988). In a two-phase system with a sharp boundary, such as dispersions of polymer latex particles, the high-q scattering decays as q^{-4} in accordance with Porod's law (Porod, 1951). The limit of the intensity in absolute units of cm^{-1} at large q can be written as

$$\lim_{q \to \infty} \left(\frac{d\Sigma}{d\Omega}\right)_{st} q^4 = 2\pi \frac{S}{V} \Delta \rho^2 \varphi (1 - \varphi), \tag{13}$$

where S is the surface area of the particles and V their volume expressed in units of cm^2 and cm^3, respectively; $\Delta \rho$ is the contrast; φ is the volume fraction of the particles.

SANS measurements were performed on a dilute suspension of monodisperse silica particles, on the instrument D22 at the ILL (Grenoble, France). The scattering from the particles was fitted to a spherical form factor, from which a mean radius of 139.0 ± 3 Å was determined. From this value, the ratio S/V in equation (13) is calculated. Assuming that the suspended silica particles are amorphous with an average mass density of 2.1 g cm^{-3}, the contrast for X-rays between silica and water is $\Delta \rho = 8.36 \times 10^{-6}$ Å^{-2} and the limit $\lim_{q \to \infty}(d\Sigma/d\Omega)_{st} q^4$ is, therefore, 1.33×10^{-5} cm^{-1} Å^{-4}.

The X-ray scattering of a 3.0% (w/w) suspension of the same silica particles in water was measured. The transmission of the suspension and its container was measured to be 0.253. The scattering of the empty capillary was subtracted from the data (Fig. 4). From a Porod representation of the data (Fig. 5) $\lim_{q \to \infty} I_S q^4$ was obtained as 1.48×10^{-8} s^{-1} Å^{-4}. Therefore, the calibration factor obtained from this measurement is

$$\lim_{q \to \infty} \left(\frac{d\Sigma}{d\Omega}\right)_{st} q^4 \frac{d_{st} T_{st+CAP}}{\lim_{q \to \infty} I_S q^4} = \frac{1.33 \times 10^{-5} \times 0.091 \times 0.253}{1.48 \times 10^{-8}}$$

$$= 20.8 \text{ s.} \tag{14}$$

Although the value obtained from this method is in very good agreement with the calibration factors obtained from the other standards, the error associated with it is significantly larger. The sources of uncertainty in this procedure arise from the determination of the particle size ($\sigma = 1\%$), the scattering length density, calculated from the density of the particles ($\sigma =$
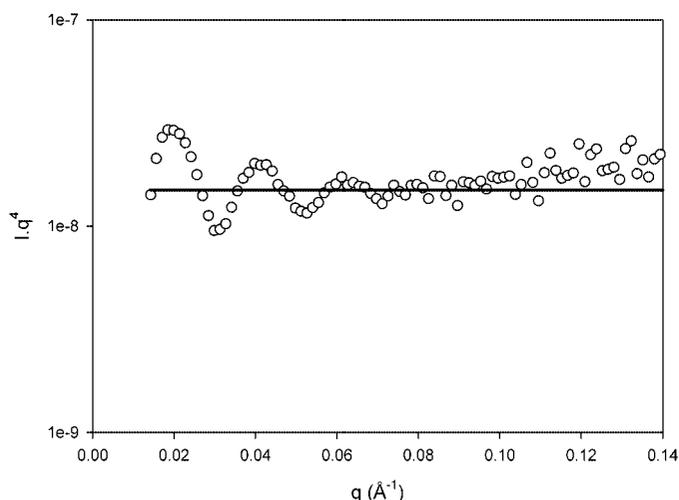
Figure 5
Porod representation of the X-ray scattering from a 3% (*w/w*) silica suspension, shown in Fig. 4.

5%), and the limiting value obtained from the Porod plot (*i.e.* the horizontal fit, to which a σ of no less than 5% is associated; this however could be reduced by further signal averaging). This gives an overall estimate of error of $\pm 14\%$ (at twice the standard deviation).

5. Discussion

This work has shown that all of the samples investigated above are suitable as calibration standards for a standard laboratory X-ray scattering instrument, provided that care is taken to measure for a long enough time and all of the sources of background scattering are accounted for. The relative merits of the standards are discussed below.

First, glassy carbon was found to be a convenient secondary standard, as reported previously (Russell *et al.*, 1988). Significantly, however, the sample has further been demonstrated, in this work, to be stable over a long time period, when stored in ambient conditions, and hence it does not need continual recalibration. The strong scattering and the small number of corrections needed during the determination greatly reduces the uncertainty in the calibration factor. Importantly, the calibration can be applied rapidly (of the order of an hour) after any modification of the setup, *e.g.* changes in the anode current or voltage, or changes in the sample–detector distance, and at regular intervals of time for routine calibration, making it an extremely convenient standard for bench-top SAXS instruments. The availability of a suitable specimen which has been previously measured (either by SANS or on a calibrated SAXS instrument), however, does provide some limitations: a point that will be addressed later.

Second, it was found that despite their very weak scattering cross sections, water and other organic solvents can be used as primary standards to calibrate a low-flux small-angle X-ray scattering instrument. Absolute calibration using water as a primary standard has been reported for a NanoStar instrument (Pedersen, 2004); however, the instrument had been optimized for weakly scattering samples, giving a flux over 50 times higher than that available on the instrument used in this study.

The pure solvents offer an alternative to standards like glassy carbon, which are not always available and require access to neutron facilities. The variation observed between the calibration factors determined for the various solvents ($\pm 5.2\%$) is within the error of absolute calibration performed at neutron facilities. It is worth noting that within the large choice of solvents available, water, despite being the most commonly used, is not the best candidate as it has a low cross section and transmission. Instead, solvents with a higher scattering cross section (*e.g.* pentane, toluene, ethanol or cyclohexane) are to be preferred as primary standards. Additionally, Zemb *et al.* have proposed the use of buffer solutions, which have larger cross sections, as alternative primary standards for SAXS calibration (Zemb *et al.*, 2003). These, however, would need to be prepared freshly, and accurately, before investigation.

Due to the combined errors associated with the evaluation of a number of parameters (transmissions, thickness of the capillary), and the subtraction of the container and dark current, significantly long signal averaging times are required to obtain a level of uncertainty which is still greater than that associated with the glassy carbon sample. This long acquisition time seriously limits the use of such samples for routine calibration at lower flux.

Third, silica suspensions offer an alternative to more common standards. Due to their high scattering, the subtraction of the empty cell is a minor source of error and good statistics can be obtained in a short period of time (*ca* 1 h). However, this requires the use of highly monodisperse suspensions, and the precise determination of the size and density of the particles, to which significant uncertainties may be associated. Indeed, the uncertainties associated with the determination of the particle density propagate significantly during the calculation of the electron density difference squared term [see equation (13)], and this needs to be determined to a high level of accuracy to reduce the overall error significantly. Moreover, silica suspensions are a less practical choice as a primary standard, as they are not stable over time and would require frequent determination of the particle size by an independent technique. A more stable, monodisperse suspension (*e.g.* a stable gold sol) (Russell *et al.*, 1988) may provide a more suitable standard, although it would still be prudent to monitor the particle size distribution over time to confirm stability, and ensure that the adsorption of particles to the wall of the capillary is not significant.

Finally, a practical and reliable approach to the routine calibration of SAXS in the laboratory would involve using a combination of the available samples. Initial calibration using the more strongly scattering of the pure solvents could be used to cross-calibrate a suitable secondary standard, such as glassy carbon, which in turn would be used for subsequent routine calibrations. This would alleviate the need for access to a SANS or high-flux SAXS facility to pre-calibrate the glassy carbon, and need only be carried out on a limited basis. To

reduce the uncertainty in this primary calibration step, it would be prudent to measure a range of pure solvents. Additionally, a freshly prepared buffer solution or freshly purchased monodisperse colloidal suspension, of known particle size and density, could also be used as an independent test of this primary calibration for greater confidence in the accuracy of the calibration of the secondary standard.

References

CRC (1983). *Handbook of Chemistry and Physics*. Boca Raton, Florida: CRC Press.

Dingenouts, N., Bolze, J., Pötschke, D. & Ballauff, M. (1999). *Adv. Polym. Sci.* **144**, 1–47.

Ghosh, R. E., Egelhaaf, S. U. & Rennie, A. R. (2000). *A Computing guide for Small Angle Scattering Experiments*, ILL Internal Report ILL98GH14T, revised.

Guinier, A. & Fournet, G. (1955). *Small Angle Scattering of X-rays*. New York: Wiley.

King, S. M. (1999). *Modern Techniques for Polymer Characterization*, edited by R. A. Pethrick & J. V. Dawkins, pp. 171–232. New York: John Wiley.

Lindner, P. (2002). *Neutrons, X-rays and Light: Scattering Methods Applied to Soft Condensed Matter*, edited by P. Lindner & Th. Zemb, pp. 23–48. Amsterdam: North-Holland Delta Series.

Orthaber, D., Bergmann, A. & Glatter, O. (2000). *J. Appl. Cryst.* **33**, 218–225.

Pedersen, J. S. (2004). *J. Appl. Cryst.* **37**, 369–380.

Porod, G. (1951). *Kolloid K.* **124**, 3–114.

Qiu, Q., Cosgrove, T., Howe, A. M. & Dreiss, C. A. (2006). *Langmuir*, **22**, 546–552.

Russell, T. P. (1983). *J. Appl. Cryst.* **16**, 473–478.

Russell, T. P., Lin, J. S., Spooner, S. & Wignall, G. D. (1988). *J. Appl. Cryst.* **21**, 629–638.

Schuster, M. & Göbel, H. (1995). *J. Phys. D Appl. Phys.* **28**, A270–A275.

Schuster, M. & Göbel, H. (1996). *J. Phys. D. Appl. Phys.* **29**, 1677–1679.

Wignall, G. D. & Bates, F. S. (1987). *J. Appl. Cryst.* **20**, 28–40.

Zemb, T., Taché, O., Né, F. & Spalla, O. (2003). *J. Appl. Cryst.* **36**, 800–805.