Absolute intensity calibration with water
**Introduction**

While for many applications it is not necessary to have the scattering data displayed on absolute scale, it does provide a useful tool to assess data quality, and it is essential for the comparison of data from different instruments, to obtain molecular weights and to determine the volume fraction of different species.\(^1\)–\(^7\) When the measured intensity is corrected for the intensity of the incoming beam, the measurement time, transmission of the sample, the volume the beam passes through, and the solid angle, the scattering cross-section of the material is what is left.

There are two intrinsically different methods to obtain scattering curves on an absolute scale. Primary calibration, based on the geometry and intensity of the beam of the used instrument,\(^8\) and secondary calibration, based on the use of a known standard. This standard can either be a primary standard of which the scattering properties can be calculated – most commonly water,\(^6\) but any other pure solvent can be used.\(^1\) Or a secondary standard, which is a previously calibrated standard, such as a protein solution of a known concentration\(^6\) or glassy carbon.\(^7\)

**Water**

Water seems to be the ideal standard for absolute intensity calibration because it is cheap and readily available in every lab. Furthermore, its scattering intensity \((I)\) can be calculated (eq. 1), which eliminates the need for a calibration measurement. The scattering intensity (often represented as the differential scattering cross-section \(d\Sigma/d\Omega\)) depends on the scattering length density \(\rho\) (\(9.388 \cdot 10^{10} \text{ cm}^{-2}\) for water) squared, the Boltzmann constant \(k_B\) (\(1.38 \cdot 10^{-23} \text{ m}^2 \text{ kg} \text{ s}^{-2} \text{ K}^{-1}\)), the temperature \(T\) in K and the isothermal compressibility \(\chi_T\) (\(10^{-10} \text{ Pa}^{-1}\) for water at 293 K).\(^9\)

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I = \frac{d\Sigma}{d\Omega} = \rho^2 k_B T \chi_T \tag{eq. 1}
\]

Often, water is assumed to give a flat curve after background subtraction, of which extrapolation of the SAXS range gives an intercept with the \(y\)-axis at an intensity of \(1.632 \cdot 10^{-2} \text{ cm}^{-1}\).\(^6\) To obtain the below depicted reduced scattering curve (Fig 1A, solid line and 1B), two measurements were done: one of the water itself (Fig 1A, interrupted line) and one of the empty holder (dotted line). Both curves have a low intensity, which hence leads to a relatively noisy result. Therefore, measurements have to be sufficiently long to obtain good statistics. Fitting a horizontal line to the reduced curve recorded at a SAXSLAB Ganesha, shows an intercept with the \(y\)-axis at \(I = 1.55 \cdot 10^{-2} \text{ cm}^{-1}\).
A closer look at the data and the fit however, reveals that the curve is not a flat line. As becomes more visible in the zoomed curve (Fig 2B), in the region around \( q = 0.4-0.6 \, \text{Å}^{-1} \) there is a slight dip in the data, this angular dependency can be explained by the fact that water actually does have a structure factor \( S(q) \neq 1 \) (Fig 2A) which is attributed to tetrahedral network formation while the slight upturn at low angles is ascribed to a combination of attractive and repulsive intermolecular interactions.\(^{10,11}\) Therefore, a polynomial fit (taking error bars on the data into account!) is better suited to fit the data, the intercept of the fitted line with the \( y \)-axis is now at \( I = 1.69 \cdot 10^{-2} \, \text{cm}^{-1} \). Figure 2A also shows that the shape of the curve is strongly affected by the temperature, as will the \( y \)-intercept, as the isothermal compressibility varies with temperature.\(^9\)

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**Fig 1.** A) Radially averaged scattering patterns of image adapted from ref 10. B) Radially averaged and background corrected scattering pattern of water at

20˚ C fitted with a horizontal line.

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**Fig 1.** A) Temperature dependency of the structure factor \( S(q) \) of water, recorded between 7 and 74˚ C, image adapted from ref 10. B) Radially averaged and background corrected scattering pattern of water at

20˚ C fitted with a polynomial fit.
Calibration

To convert your data to absolute intensity, you need to determine the calibration factor. This is done by measuring a water sample and the empty holder, after standard reduction of the data the empty holder can be subtracted from the water measurement. The scaling factor is obtained by taking the ratio of $1.632 \cdot 10^{-2}$ cm$^{-1}$ and the result of fitting the data and extrapolating to $q = 0$. This scaling factor is the correction factor for the samples. Not coincidentally, intensity for data measured on a SAXSLAB instrument lies within 5% of the value for water which is within the experimental uncertainty.

Concluding remarks

Although water is a well-known, and often used standard for intensity calibration of X-ray scattering data, it is not ideal for the calibration of lab-instruments. Firstly it requires long measurements to obtain good statistics. Secondly, fitting the data might be affected by the presence of a structure factor. If a primary standard is preferred, it is advisable to use solvent with a higher scattering cross-section and higher transmission such as for example ethanol ($d\Sigma/d\Omega = 2.58 \cdot 10^{-2}$ cm$^{-1}$).$^1$

References and further reading