4 Analysis of the small-angle X-ray and neutron scattering spectra

Small-angle X-ray and neutron scattering and their applications have been subject of many publications, equations not specifically cited can be found in [31,57,123].

4.1 SAXS analysis

The experimental solute scattering curve, $I_{EXP\,SAXS}(Q, c)$, was found from the experimental D_2O and sample scattering patterns as follows:

$$I_{EXP\,SAXS}(Q, c)=\{I_{S/SAXS}(Q, c) - I_{D2O/SAXS}(Q)\}\times f_{H2O}/\{c\times N_a/M\} \quad (4.1)$$

where N_a is Avogadro's number, M is the molecular mass of the solute molecule [g/mol], c is the solute concentration, g/mL. The factor f_{H2O} is arising due to normalization to the scattering by H_2O [97] and given by:

$$f_{H2O} = I_{H2O}(0)/(I_{H2O} - I_{EC}) \quad (4.2)$$

$I_{H2O}(0)$, the zero-angle X-ray scattering of H_2O, is 0.01632 cm^{-1} at 20 °C [97]. The denominator in eq. (4.2) is the difference in the experimentally measured scattering intensity between the H_2O filled (I_{H2O}) and empty cell (I_{EC}) and provides the scattering by H_2O. Only I_{H2O} was measured for the employed cells; the ratio I_{H2O}/I_{EC} was known to be ≈ 1.34±0.05, and thus one could evaluate f_{H2O} that was necessary to put the data on an absolute scale. In the following $I_{EXP\,SAXS}(Q, c)$ has dimension of barn, 1 barn = 10^{-24} cm^2.

The orientationally averaged self-convolution of the excess electron density is the *correlation function* $\gamma(r)$:

$$\gamma(r) = <\int\Delta\rho(\boldsymbol{u})\Delta\rho(\mathbf{u}+\boldsymbol{r})d\mathbf{u}>_\Omega \quad (4.3)$$

where $<>_\Omega$ denotes averaging over the solid angle Ω and the integration is performed over the volume of the particle. $\Delta\rho(\boldsymbol{r})$ is the excess electron density, $\Delta\rho(\boldsymbol{r}) = \rho(\boldsymbol{r}) - \rho_0$, with $\rho(\boldsymbol{r})$ and ρ_0 being electron densities of the solute and solvent, respectively. The contrast, $\Delta\rho_{AV} = \rho_{AV} - \rho_0$, is the average value of $\Delta\rho(\boldsymbol{r})$, ρ_{AV} is the average electron density of a particle. The scattering from the solute molecule, $I(Q)$, is the Fourier transform of $\gamma(r)$:

$$I(Q)=4\pi\int_0^\infty \gamma(r)\frac{\sin Qr}{Qr}r^2 dr \quad (4.4)$$

where $\boldsymbol{Q}$ is the vector of reciprocal space (Q being its modulus). The theoretical SAXS curve, $I_{THEO\,SAXS}(Q)$, and the theoretical correlation function, $\gamma_{THEO}(r)$, were computed from the known atomic structures of cyclodextrins studied here [1,4,6,24] using a set of programs in which the cube method [30,89] was implemented.

In short, the solute molecule is inscribed into a parallelepiped which is subdivided into

small cubes with edges of 0.2-0.6 Å. The distance between the center of the atom belonging to the molecule (with coordinates x, y, z) and the center of the *i*th cube (with coordinates xc_i, yc_i, zc_i) is evaluated as $\sqrt{\{(xc_i-x)^2 + (yc_i-y)^2 + (zc_i-z)^2\}}$ and then compared to the van der Waals radius of this atom. If at least for one atom belonging to the molecule, the value of the van der Waals radius (taken from [104]) is greater than the distance between the center of the cube and center of the atom, the cube is attributed to the solute molecule, otherwise it is attributed to solvent. (Some of the cubes attributed by this procedure to solvent are in fact within the excluded volume. Such cubes are re-attributed to the solute molecule by an auxiliary computational procedure.)

Thus, the integral (4.3) becomes a summation over all the cubes belonging to the solute molecule with $\Delta\rho(\boldsymbol{r})=\Delta\rho_i$, *i* denotes an *i*th cube. In the "homogeneous approximation" (i.e. assuming the electron density of the solute molecule to be uniformly distributed over its volume), $\Delta\rho_i$ is equal to $\Delta\rho_{AV}$ if the *i*th cube belongs to the particle, and 0 if it does not. Application of this approximation allowed to compute $\gamma_{THEO}(r)$ and the theoretical SAXS curve from eqs. (4.3, 4.4). Further, the SAXS curve was multiplied by r_0^2, where $r_0 = 0.282\times10^{-12}$ cm is the scattering length of one electron; the theoretical SAXS curve is therefore on the same scale as $I_{EXP\,SAXS}(Q, c)$ obtained from eq. (4.1).

The maximum diameter of a particle, D, was estimated from the spatial cosine transform of $I_{EXP\,SAXS}(Q, c)$:

$$\Phi(\acute{o})=\int_0^{\infty} I_{EXP\,SAXS}(Q, c)\cos(Q\acute{o})\,dQ \qquad (4.5)$$

under the assumption that $\Phi(\acute{o})$ is zero for $\acute{o} > D$ ([90], p. 82 in [31]). The values of D_{EXP} were compared to D_{THEO} values, the latter being determined by $\gamma_{THEO}(r)$ under consideration that $\gamma_{THEO}(D_{THEO})\equiv0$. The lowest and the upper integration limits in eq. (4.5) were equal to 0 and 0.5 Å^{-1}, respectively. (For $Q < 0.05$ Å^{-1}, $I_{EXP\,SAXS}(Q, c)$ was found from eq. (4.6).)

The initial part of the experimental SAXS curve (0.05 Å^{-1} < Q < 0.2 Å^{-1}) was used to evaluate experimental values of intensity at the origin, $I_{EXP\,SAXS}(0)$, and the square of the radius of gyration, $R^2_{g(EXP)}$, according to Guinier's relation:

$$I_{EXP\,SAXS}(Q) = I_{EXP\,SAXS}(0)\times\exp(-R^2_{g(EXP)}Q^2/3) \qquad (4.6)$$

The value of the square of the radius of gyration in the "homogeneous approximation", $R^2_{g(THEO)}$, was found (see e.g. [31]) as follows:

$$R^2_{g(THEO)}=\frac{1}{2}\int_0^D \gamma_{THEO}(r)r^4\,dr \Big/ \int_0^D \gamma_{THEO}(r)r^2\,dr \qquad (4.7)$$

The volume of the homogeneous partide, V, is:

$$V = 2\pi^2 I(0) / \int_0^\infty I(Q) Q^2 dQ \quad (4.8)$$

A value of volume found according to eq. (4.8), but with the upper integral limit being Q_{max}, will be denoted by $V(Q_{max})$; obviously, for $Q_{max} \to \infty$, $V(Q_{max}) \to V$. One evaluates $V_{EXP}(Q_{max})$ and $V_{THEO}(Q_{max})$ from $I_{EXP\ SAXS}(Q)$ and $I_{THEO\ SAXS}(Q)$, respectively. The value of V_{EXP} was found as follows:

$$V_{EXP} = V_{THEO} \times V_{EXP}(Q_{max}) / V_{THEO}(Q_{max}) \quad (4.9)$$

Because the spectrum $I_{D2O}(Q)$ changes slightly during the experiments (by a factor from 0.9 to 1.1 for $Q > 0.05$ Å^{-1}), even two SAXS curves for the same solution are eventually shifted with respect to each other by an additive constant (that is: $I_{EXP\ SAXS}(Q, c)|_{t1} = I_{EXP\ SAXS}(Q, c)|_{t2} + const$, where "t1" and "t2" refer to the SAXS patterns of the same solution taken at different times). Therefore, for the comparison of a series of curves obtained for different concentrations, the curves were shifted (by an appropriate choice of the constant *const*) so that all of them coincided for $Q \geq 0.45$ Å^{-1}. Such a manipulation is justified if the intermolecular structure factor is close to the unity in this Q region (as, for instance, was found for γ-CD). For DIMEB and TRIMEG, $S_{SOL}(Q, c)$ became somewhat less than unity (for $Q \geq 0.45$) upon increase of the concentration, but the error introduced through the shift of curves was smaller than the error that would have occurred if no shift was performed.

4.2 SANS analysis

The theoretical small-angle neutron scattering curve, $I_{THEO\ SANS}(Q)$, was computed as described in [30,89]:

$$I_{THEO\ SANS}(Q) = \langle |F(\boldsymbol{Q}) - \zeta(\boldsymbol{Q})|^2 \rangle_\Omega \quad (4.10)$$

by using the cube method (see above). $F(\boldsymbol{Q})$ is the scattering amplitude of the solute molecule of volume V in vacuum, and $\zeta(\boldsymbol{Q})$ is the scattering amplitude of the volume V filled with solvent with scattering density ρ_0:

$$F(\boldsymbol{Q}) = \sum_{i=1}^{n} \langle b \rangle_i \exp(-i\,\boldsymbol{Q}.\boldsymbol{r}_i) \quad (4.11)$$

$$\zeta(\boldsymbol{Q}) = \rho_0 \int_V \exp(-i\,\boldsymbol{Q}.\boldsymbol{r})\, d\boldsymbol{r} \quad (4.12)$$

where $\langle b \rangle_i$ is the coherent scattering length of the ith atom [cm], and n is the number of atoms in the solute molecule. The scattering amplitude of the excluded volume, $\zeta(\boldsymbol{Q})$, was evaluated using the value of the scattering length density of heavy water, $\rho_0 = 0.064 \times 10^{-12}$ cm^{-2} ($\rho_0 = \{2\langle b \rangle_D + \langle b \rangle_O\}/V_{D2O}$, where V_{D2O} is the volume of the D_2O molecule). It is worth to emphasize, that in evaluation of $F(\boldsymbol{Q})$ for neutron scattering, no approximation is involved. On the contrary,

for SAXS, the "homogeneous approximation" corresponds to:

$$F_{SAXS}(\boldsymbol{Q}) = \int_V \rho(\boldsymbol{r}) \exp(-i\,\boldsymbol{Q}.\boldsymbol{r})\, d\,\boldsymbol{r} \approx \int_V \rho_{AV} \exp(-i\,\boldsymbol{Q}.\boldsymbol{r})\, d\,\boldsymbol{r} \tag{4.13}$$

The experimental SANS curve $I_{EXP\,SANS}(Q, c)$ [barn], corrected for incoherent background (I_{INC}) and concentration c [g/mL] was found from the experimental SANS pattern $I_{S/SANS}(Q, c)$:

$$I_{EXP\,SANS}(Q, c) = I_{S/SANS}(Q, c)/(c \times N_a/M) - I_{INC} \tag{4.14}$$

Note that $I_{S/SANS}(Q, c)$ is already normalized to H_2O scattering (see section 3.4).

4.3 Intermolecular structure factor $S_{SOL}(Q)$

When the scattering curve is solely due to particle scattering in a very dilute solution, $I(Q)$ is given by eq. (4.10). For solutions at concentration c, the expression for $I(Q, c)$ reads:

$$I(Q, c) = S_{SOL}(Q, c) \times I(Q) \tag{4.15}$$

where $S_{SOL}(Q, c)$ is the intermolecular structure factor, see sections 2.3-2.4. Eq. (4.15) holds under the condition:

$$\langle|F(\boldsymbol{Q}) - \zeta(\boldsymbol{Q})|^2\rangle_\Omega \approx \langle|F(\boldsymbol{Q}) - \zeta(\boldsymbol{Q})|\rangle_\Omega^2 \tag{4.16}$$

in particular for CDs and mCDs it holds for $Q < 0.45$ Å^{-1}. More generally, one can write:

$$I(Q, c) = \gamma_{CM\,SOL}(Q, c) \times \langle|F(\boldsymbol{Q}) - \zeta(\boldsymbol{Q})|\rangle_\Omega^2 + I(Q) \tag{4.17}$$

where $\gamma_{CM\,SOL}(Q, c)$ is related to the intermolecular structure factor (see section 2.4) as follows:

$$\gamma_{CM\,SOL}(Q, c) = S_{SOL}(Q, c) - 1 \tag{4.18}$$