

8 Conclusions

8.1 Small-angle X-ray and neutron scattering

In the present work the first small-angle X-ray scattering (SAXS) measurements on aqueous solutions of cyclodextrins (CDs), β - and γ -CD, and the methylated cyclodextrins (mCDs), per-dimethylated β -CD (DIMEB) and per-trimethylated γ -CD (TRIMEG), and the first small-angle neutron scattering (SANS) measurements on solutions of DIMEB are reported. Because of the relatively small molecular masses (1-1.6 kDa), CDs and mCDs belong to the smallest molecules studied by small-angle scattering. Nevertheless, the recorded scattering patterns could be successfully analyzed, and the main results of the analyses are summarized below.

The SAXS curves of the dilute solutions of the four compounds were put on an absolute scale and compared to the scattering curves computed from the corresponding crystallographic structures under application of the “homogeneous approximation”. A good agreement was found for β - and γ -CD whereas the measured scattered X-ray intensity from DIMEB and TRIMEG solutions was quite different from the computed intensity: smaller in the low Q region ($Q < 0.2 \text{ \AA}^{-1}$) and larger in the high Q region ($Q > 0.3 \text{ \AA}^{-1}$) studied.

The maximum diameter, the square of the gyration radius and the volume of the solute molecules were evaluated from the scattering by dilute solutions of CDs and mCDs and compared to the corresponding calculated values. Here again, a good agreement was found for β - and γ -CD, whereas for DIMEB and TRIMEG the calculated values were larger than the experimental ones, which is consistent with the differences observed between the computed and experimental SAXS curves of mCDs.

One may conclude that under the conditions of this study, β - and γ -CD are present in solution only as monomers and that the small-angle X-ray scattering is primarily due to “shape scattering” (i.e. the electron density distribution is rather homogeneous). For DIMEB and TRIMEG, the poor agreement between values found from the SAXS experiment and their corresponding theoretical predictions indicates, most likely, the failure of the “homogeneous approximation”.

This failure is caused by the presence of methyl groups leading to a substantially smaller electron density on the periphery than in the inner part of the molecule. Therefore, the inhomogeneities in the electron density distribution (and the extent of their contribution to the SAXS curves) is greater for methylated CDs, as opposed to native CDs. Consistent with this are

also the greater discrepancies (between the experiment and calculations) found for TRIMEG as compared to DIMEB, which is in agreement with the fact that the former has more methyl groups (and thus a greater extent of inhomogeneities) than the latter. The experimental SAXS intensity from dilute aqueous solutions of mCDs for $Q > 0.3 \text{ \AA}^{-1}$ is higher than the intensity computed using the “homogeneous approximation”, confirming the presence of the monomeric form only.

The influence of temperature and concentration on the SAXS curves of β -CD, γ -CD, DIMEB and TRIMEG as well as the temperature dependence of the SANS pattern of DIMEB were studied. No significant change with temperature for the β -CD curve was seen. The change of the γ -CD scattering curves with increasing temperature and/or concentration indicated the presence of repulsive interactions. For DIMEB, the presence of attractive intermolecular interactions has been observed in all experimental SAXS curves and experimental SANS patterns. On the contrary, TRIMEG solutions showed net repulsive interactions at room temperature and with increasing temperature the net interactions became more attractive. This agrees with the fact that, while both mCD exhibit negative temperature coefficient of the solubility in water, TRIMEG crystallizes at much (10-20 °C) higher temperature than DIMEB.

The SANS and SAXS results for aqueous DIMEB solutions were in agreement. Under the assumption that the SANS curve computed for DIMEB is correct, the experimental SANS curves indicate that even for a DIMEB concentration of 6.6 mg/mL in D₂O and at room temperature, the solute-solute interactions are substantial and cannot be neglected.

8.2 Quasielastic neutron scattering (QENS) results

In the frame of the performed investigations, QENS spectra of D₂O and H₂O solutions of β - and γ -CD, DIMEB and TRIMEG were recorded and analyzed; only the results for D₂O solutions are included in the present work.

The analysis of the high energy resolution spectra of DIMEB ($\Delta E \approx 1 \text{ \mu eV}$) under neglect of rotational motion yields values of the solute translational diffusion coefficients ($D_{\text{TR SOL}}$) that are close to the ones determined by PFG-NMR measurements. Apparently, the relative contribution of the translational component was appreciably increased; the reason for this was attributed to the presence of attractive solute-solute interactions and a related increase of $S_{\text{SOL}}(Q)$ in the low Q region. The temperature dependence of the slight difference between QENS and PFG-NMR $D_{\text{TR SOL}}$ -values could be tentatively explained on the basis of variation of $S_{\text{SOL}}(Q)$ with temperature and concentration as observed by SAXS.

An attempt to fit the “standard solute model” (which includes rotational and translational motion of the solute) could not provide a satisfactory description of the DIMEB

spectra recorded with the energy resolution of $\Delta E \approx 30 \mu\text{eV}$. A need for the introduction of an additional “broad” scattering component (with the intrinsic width from 0.2 meV to 0.5 meV) was recognized. The intensity of this component was found to correspond to a scattering cross section in the range of 3700 barn - 7500 barn and to increase with Q and with temperature.

This “broad” component was ascribed to a restricted (probably rotational) motion of $-\text{CH}_3$ and $-\text{CH}_2\text{-O-CH}_3$ groups. This interpretation is based on the fact that the hydrogen atoms of $-\text{CH}_3$ groups alone have a total scattering cross section of about 3500 barn. The description of the “broad” scattering component of DIMEB by the model of jumps over three equivalent sites on a circle resulted in a time between successive jumps of about 3 ps to 7 ps and in a value in the range of 3 Å - 7 Å for the radius of a circle. QENS spectra with Q values up to 4 \AA^{-1} and with ΔE values of $30 \mu\text{eV}$ - $50 \mu\text{eV}$ will be required to obtain more precise information.

So far, unfortunately, no $\Delta E \approx 30 \mu\text{eV}$ spectra of D₂O solutions of other mCDs are available to search for a possible correlation between the intensity of the “broad” component and the number of methyl groups and glucose residues. For the same reason, the tests whether such a “broad” component appears also in the spectra of γ -CD (β -CD is only poorly soluble in D₂O) could not be performed.

The application of the “standard solute model” to DIMEB D₂O solutions provided the value of the solute rotational diffusion coefficient $D_{\text{r SOL}} = 0.26 \mu\text{eV}$ at 7 °C. After the amendment of the model with an additional “broad” component, smaller $D_{\text{r SOL}}$ ($\approx 0.13 \mu\text{eV}$ at 7 °C) values were obtained, in qualitative agreement with the literature values for β -CD (e.g. $\approx 0.16 \mu\text{eV}$ at 0 °C in H₂O solution). Interestingly, the value of $D_{\text{r SOL}}$ of DIMEB in D₂O solution was found to drop at temperatures above 40 °C in the fits to the $30 \mu\text{eV}$ and $90 \mu\text{eV}$ spectra. To confirm these results, an analysis of $30 \mu\text{eV}$ spectra of γ -CD, for which a continuous increase of $D_{\text{r SOL}}$ with temperature is certain, would be of advantage.

In the spectra of mCD solutions recorded with the energy resolution of 1, 10, 30 and 90 μeV , a higher, than predicted by the “standard solute model”, intensity of the solute component was observed in the low Q region ($Q < 0.5 \text{ \AA}^{-1}$). Such a discrepancy can be explained, in part, by the increase of $S_{\text{SOL}}(Q)$ towards low Q as observed in SAXS/SANS. This increase was neglected in the “standard solute model”, where the approximation $S_{\text{SOL}}(Q) = 1$ was employed. However, in the case of the especially low Q range of the spectra measured with 10 μeV resolution, the intensity of the solute component is by the factor ≈ 7 higher (judging by the value of the scaling factor), than predicted by the “standard solute model” (disregarding whether the motion of $-\text{CH}_3$ and $-\text{CH}_2\text{-O-CH}_3$ groups is taken into account). The increase of $S_{\text{SOL}}(Q)$ with decreasing Q cannot account for this, since the values of $S_{\text{SOL}}(Q)$ obtained from the small-angle scattering experiments were smaller than 2 in the whole Q range studied.

In order to explain the origin of the high intensity of the solute component, it was assumed that part of this intensity comes from the coherent scattering contribution of the heavy water molecules in the hydration shell of the mCD. Such an assumption is in accord with the fact that the quasielastic coherent scattering can originate from the correlations between positions of the solute and solvent molecules. The model was developed, where the hydration water molecules were assumed to diffuse together with a mCD molecule and therefore to give a rise to the scattering component with an intrinsic width equal to the width of the solute scattering component. This model (“hydrated solute model”) was fitted to the spectra and the number of water molecules in the hydration shell was found to be about 60 per DIMEB-molecule and 70 per TRIMEG-molecule.

The elastic coherent structure factor of the hydration shell of mCDs (evaluated in the frame of the “hydrated solute model”) decreases fast with Q , it is appreciable only for $Q < 0.4 \text{ \AA}^{-1}$. Thus, QENS spectra recorded with a long incident wavelength are required for further investigations. In addition, the increase of $S_{\text{SOL}}(Q)$ towards low Q must be accounted for quantitatively, to make a precise evaluation of the coherent scattering due to hydration shell possible. (In the present work the approximation $S_{\text{SOL}}(Q) = 1$ was employed.)

So far, an analysis of the QENS spectra of β -CD in D_2O solution didn't furnish many results; the main difficulty is the low solubility of β -CD and the related low solute concentration in the measured solutions. The values of translational diffusion coefficients were determined from 1 \mu eV spectra and found to be higher than the values found in the other studies (e.g. [75,98]).

For γ -CD and TRIMEG, the absence of $\Delta E = 1 \text{ \mu eV}$ and 30 \mu eV spectra of D_2O solution and the lack of $D_{\text{TR SOL}}$ values makes the detailed analysis of the available $\Delta E = 10 \text{ \mu eV}$ and 90 \mu eV spectra difficult.

8.3 Conclusions on the analysis of QENS spectra of aqueous CDs/mCDs solutions

Owing to the high incoherent cross section of the hydrogen atom, usually, it is the QENS spectra of H_2O solutions, which are analyzed with the goal to elucidate properties of the hydration shell. Because of the large contribution from the bulk H_2O , rather high solute concentrations are usually employed in order to make the scattering due to the hydration shell stronger. However, even if the scattering by the bulk H_2O is neglected (which is sometimes done at high solute concentrations, when all water is assumed to be “hydration water”), one will have to discriminate between the motion of the water molecules in the hydration shell and the motion of the solute molecule.

The QENS spectra of solutions in D_2O provide very useful information, because the

scattering of the solute is relatively intense, and can be used to determine dynamical and structural parameters of the solute molecule, which can be further applied (with eventual corrections) in the analysis of the spectra of H₂O solutions.

An analysis of the QENS spectra of solutions in D₂O will generally require consideration of the coherent scattering contribution. It was shown in the present work that taking into account *at least* the intramolecular coherent scattering (through the rotational structure factors $A_i(Q)$, in particular through the elastic structure factor (ESF), $A_0(Q)$), is straightforward and should in fact always be done, given that the (crystal) structure of the solute is available. An account for the intermolecular coherent scattering due to solute-solute spatial correlations requires the knowledge of the intermolecular structure factor, $S_{\text{SOL}}(Q)$. As shown in the present work, SANS and SAXS measurements not only give interesting results *per se*, but also yield the quantitative information required for the interpretation of QENS results, namely $S_{\text{SOL}}(Q)$ as a function of temperature and solute concentration.

The quasielastic coherent scattering in D₂O solution contains also information on the spatial correlations between the solvent and the solute molecules. For the case of aqueous solutions it was shown that it is possible in principle to obtain the number of water molecules in the hydration shell. A more sophisticated theoretical model should allow to extract the average time spent by the water molecule in the hydration shell.

8.4 Summary of the results on aqueous solutions of methylated CDs

The following experimental results obtained in the present work are of interest with respect to the unusual temperature dependence of the solubility of methylated cyclodextrins.

1. The interactions between mCD molecules in solution become stronger with increasing temperature.
2. the onset (near 35 °C) of a decrease of the DIMEB rotational diffusion coefficient ($D_{\text{r SOL}}$) in D₂O solution with increasing temperature, which is probably related to the stronger attractive interactions between DIMEB molecules.
3. A determination of the number N_{HYD} of water molecules in the hydration shell as a function of temperature was achieved for DIMEB and TRIMEG. Apparently, the value of N_{HYD} decreases with increasing temperature in the case of DIMEB.
4. in DIMEB solutions the motion of -CH₃ and -CH₂-O-CH₃ groups becomes faster with temperature, possibly contributing to the disruption of the hydration shell (and to the decrease of N_{HYD}) at higher temperatures.
5. no oligomers/aggregates were found in aqueous solutions of mCDs at least up to 5 °C before crystallization takes place.

8.5 Outlook

Additional small-angle X-ray and/or neutron scattering measurements are necessary in order to obtain $S_{\text{SOL}}(Q)$ for temperatures in the range from 5 to 50 – 70 °C and for different concentrations of CDs and mCDs. The knowledge of $S_{\text{SOL}}(Q)$ applied in the analysis of the QENS spectra would allow to test more thoroughly the hypothesis concerning the coherent scattering from the hydration shell and to further confirm whether, indeed, it is because of the attractive interactions, that the $D_{\text{TR SOL}}$ values obtained in QENS are close to those found in PFG-NMR. QENS spectra with intermediate energy resolution and the broad Q range for DIMEB, γ -CD and TRIMEG are required for the further investigation of the essence of the “broad” scattering component with $\text{FWHM} \approx 0.5$ meV, tentatively attributed to the motion of $-\text{CH}_3$ and $-\text{CH}_2\text{-O-CH}_3$ groups, and the temperature dependence of $D_{\text{r SOL}}$.