1 Introduction

1.1 The structure of cyclodextrins

The α-, β- and γ-cyclodextrins (CDs) are cyclic oligosaccharides consisting of 6, 7 and 8 D-glucose units, respectively. They are well known for their ability to form inclusion complexes with molecules that can fit (completely or partially) into their central cavity [107, 126,128]. There is a vast amount of literature concerning applications of cyclodextrins and their derivatives in pharmacy, chromatography, food and cosmetics industry [127,128]. Although there are also cyclodextrins built of 9 and more glucose residues (see e.g. [108]), throughout this work when speaking of cyclodextrins (or native cyclodextrins) only α-, β- and γ-CD are referred to.

In cyclodextrins glucose (more precisely, glucopyranose) units in $^4C_1$- chair conformation are linked through α(1→4) bonds (Fig. 1.1). The glucoses are *syn* oriented, i.e. their O6 hydroxyls are on one side of the ring and their O2, O3 hydroxyls on the other side. The round conformation of the CD macrocycle is stabilized through the intramolecular interglucose O2(n)...O3(n-1) hydrogen bonds (Figs. 1.1, 1.2).

The geometrical form of a cyclodextrin molecule can be described as a truncated hollow cone, it is close to a torus, as seen from Fig. 1.3. The external surface of the macrocycle is hydrophilic due to the presence of hydroxyl groups and the surface inside the cavity is hydrophobic. Approximate dimensions of the cyclodextrin macrocycles are listed in Tab. 1.1.

Table 1.1 **Characteristics of α-, β- and γ-CD.** [127,128]

	α-CD	β-CD	γ-CD
Number of glucose units	6	7	8
Molecular weight	972	1135	1297
Solubility in water at room temperature [g/L]	145	18.5	232
Cavity diameter [Å]	4.7-5.3	6.0-6.5	7.5-8.3
Height of torus [Å]	7.9±0.1	7.9±0.1	7.9±0.1
Diameter of outer periphery [Å]	14.6±0.4	15.4±0.4	17.5±0.4
Approximate volume of the cavity [Å^3]	174	262	427
Approximate total volume [Å^3][a]	1149	1209	1473

[a] Evaluated from the data given in the present table.

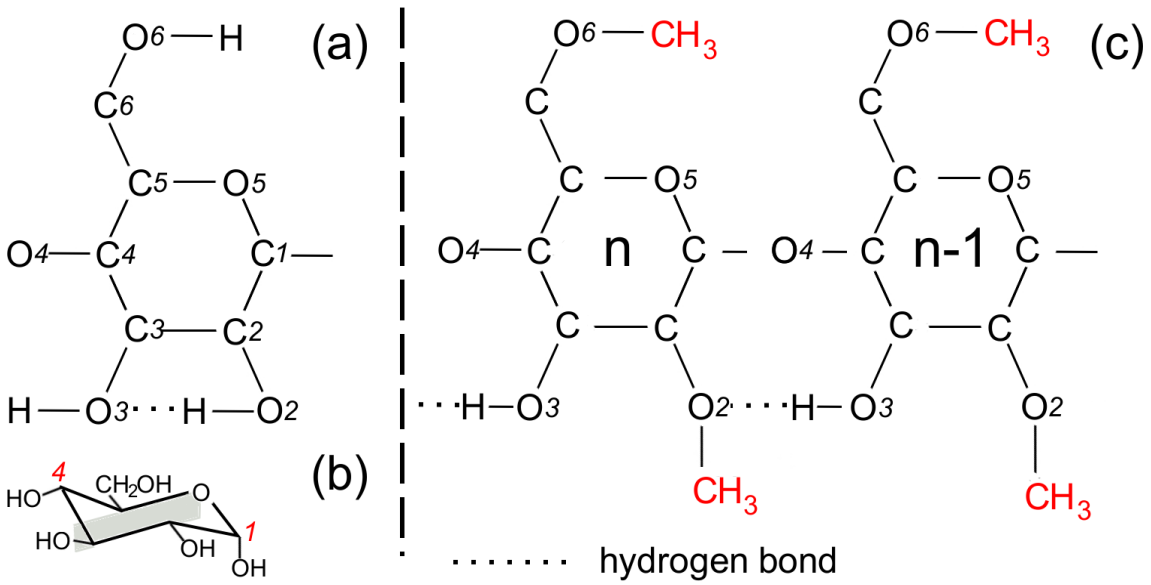

Figure 1.1 **The glucose residue in cyclodextrins. (a):** the numbering scheme of a glucose unit; **(b):** the $^4C_1$ "chair" conformation adopted by glucose in the CD macrocycle; **(c):** two adjacent glucoses of per-(2,6-di-O-methyl)-β-cyclodextrin (DIMEB). In DIMEB and native cyclodextrins O3-H···O2 hydrogen bonds stabilize the "round" structure of the macrocycle. In per-trimethylated cyclodextrins (e.g. per-(2,3,6-tri-O-methyl)-γ-cyclodextrin, TRIMEG), O3-H···O2 hydrogen bonds are not possible (O3-H is methylated), giving rise to more structural flexibility of the macrocycle.

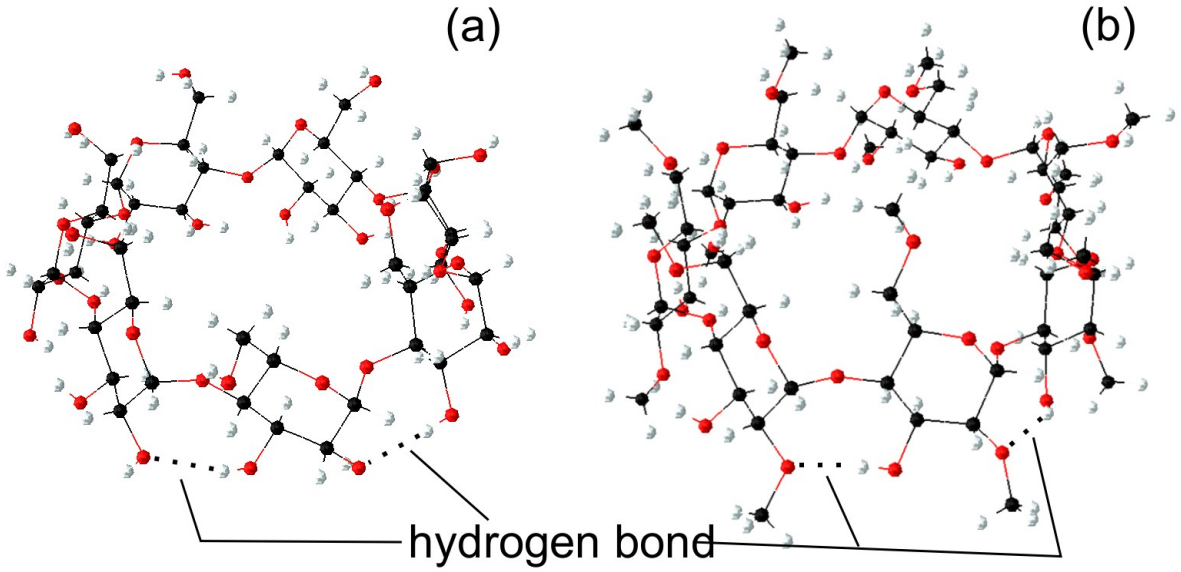

Figure 1.2 **Crystallographic structures of β-CD and per(2,6-O-methyl)-β-CD (DIMEB). (a):** structure of β-CD [6]; **(b):** structure of DIMEB [1]. Carbon, oxygen and hydrogen atoms are black, red and light blue, respectively. Hydrogen bonds O2(n)...O3(n-1) stabilize the "round" structure of the macrocycle (two of such bonds are indicated by dots).

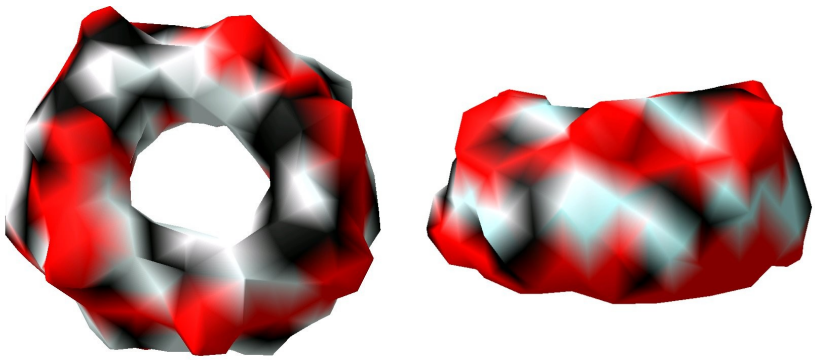

Figure 1.3 **The geometry of the β-CD macrocycle.** The structure was taken from [6]; the molecular surface was computed by the program SPDBV [42].

Note that β-CD has much lower aqueous solubility than α- and γ-CD (Tab. 1.1). The results of computer simulations [72,91] explain this phenomenon by the strong water ordering around the molecule of β-CD in aqueous solution.

The properties of CDs and their inclusion complexes were studied by means of molecular modeling by F. W. Lichtenthaler and S. Immel, see for instance [70,71]. The large list of references to the solid state structures of cyclodextrins, their derivatives and inclusion complexes can be found on the homepage of S. Immel [50].

1.2 Negative temperature coefficient of the solubility of methylated cyclodextrins

The native CDs show positive temperature solubility coefficients in water, i.e. their solubility in water rises with temperature [37,53,55,145,146]. If the glucoses of CDs are per-dimethylated at all their O2 and O6 or per-trimethylated at all their O2, O3 and O6 hydroxyl groups (see Figs. 1.1 and 1.2 where the structure of a per-dimethylated derivative is shown), the temperature coefficient of the solubility of resulting derivatives in water becomes negative. Specifically, for per(2,6-di-O-methyl)-β-cyclodextrin (DIMEB) the solubility in water at room temperature is ca. 0.6 g/mL, for increasing temperature it gradually reduces and falls to values <0.01 g/mL for $t > 70$ °C [45,140].

(The abbreviations employed for the designation of methylated cyclodextrins are listed in Tab. 1.2.)

Table 1.2 **The abbreviations employed for the designation of per-dimethylated and per-trimethylated cyclodextrins.**

	# = α-CD	# = β-CD	# = γ-CD
per(2,6-di-O-methyl)-#	DIMEA	DIMEB	DIMEG
per(2,3,6-tri-O-methyl)-#	TRIMEA	TRIMEB	TRIMEG

In cold water, methylated cyclodextrins (mCDs) are even more soluble than native CDs, but they crystallize from the aqueous solution upon heating, either as crystallohydrates with only few water molecules or in the anhydrate form. On the contrary, crystals of mCDs grown at low temperature are always substantially more hydrated.

The following methylated cyclodextrins were crystallized upon heating: i) as anhydrate: DIMEA [117], DIMEB [115], TRIMEA [116]; ii) as monohydrate: TRIMEB · H_2O [16]; iii) as dihydrate: TRIMEG · $2H_2O$ [118]. At low temperatures were grown: (4TRIMEG) · 19.3 H_2O [2], TRIMEG · 4.5 H_2O [4], DIMEB · $15H_2O$ [3]. It was suggested [3,4] that the structures of DIMEB · $15H_2O$ and (4TRIMEG) · 19.3 H_2O reflect the existence of a well ordered hydration shell in cold aqueous solutions of DIMEB and TRIMEG. As predicted by the hydrophobic effect, such an ordering would become energetically unfavorable upon increase of the temperature, resulting in the aggregation and finally crystallization of the solute.

It is not clear, what happens to the hydration shell when the solution is heated. A hypothesis was made [3,4], that with rising temperature water molecules would become more mobile and the macrocycle more flexible. This would lead to the break down of the hydration shell and to crystallization of methylated CDs with few or no water molecules at all.

Monte Carlo and molecular dynamics simulations were made on aqueous solutions of β-CD and DIMEB at 25 and 70 °C [114]. It was found that at 70 °C β-CD becomes better integrated in the water hydrogen-bonded network. On the contrary, for DIMEB molecule at 70 °C the number of water molecules near hydroxyl groups had decreased (being 46 per DIMEB molecule at 25 °C and only 31 at 70 °C), and the number of water molecules near methyl groups had decreased drastically (being 147 per DIMEB molecule at 25 °C and only 18 at 70 °C).

1.3 The motivation of this study

The negative temperature coefficient of the solubility observed for the aqueous solutions of methylated cyclodextrins is the manifestation of the hydrophobic effect [131]. A detailed understanding of the nature of this effect is important for protein science and, consequently, for the progress in medicine, biosciences and biotechnology.

Even the smallest proteins are significantly more complex molecules than cyclodextrins. The latter, therefore, represent a simpler system for the investigation of the structure and dynamics of the hydration shell, aiming at a better understanding of the hydrophobic effect. It was the *crystal structures* obtained for native cyclodextrins and their methylated derivatives, which led to the hypothesis about the changes in hydration shell of mCDs upon increase of the temperature [3,4,5]. Naturally, this hypothesis called for an investigation of the properties of the

aqueous solutions of native and methylated CDs. Such an investigation was started in 1999 by T. Aree [5] and continued in the frame of the present work.

The quasielastic neutron scattering (QENS) technique probes the microscopic structure and dynamics, allowing, in principle, the direct observation of the structure and dynamics of the hydration shell. The small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) methods provide information on the structure of the solute molecule in solution (as opposed to the crystal structures) and the solute-solute interactions. The combined application of QENS, SAXS and SANS makes it possible to gain a deep insight into the structure of aqueous solutions of CDs and mCDs and into the dynamics of both solute and water molecules. Moreover, the results obtained and the methodology developed should in principle be suitable for a later application in the investigations of the properties of protein sdutions.

The examples of applications of quasielastic neutron scattering for the investigation of aqueous solutions are not numerous. The main reason is probably the limited amount of available measurement time at a relatively small number of neutron spectrometers which are suitable for QENS experiments. A non-exhaustive list of examples of QENS studies carried out in the past on aqueous solutions of substances, relevant to the studies of hydration shell of biomolecules, comprises: trimethyl-amino-N-oxide [17], tert-butyl alcohol [17,18], fructose [29], glucose [130], trehalose, maltose, sucrose [14,78,79,80], vitamin C [82,83], myoglobin [74], bovine serum albumin [28] and N-acetyl-leucine-methylamide [106].

The analysis of the QENS spectra recorded in the frame of these studies is in many cases less complete than it could be. For instance, the contribution of coherent neutron scattering is often neglected, although it can in principle be accounted for (at least partially) on the basis of available data from other sources. Another example: the rotational motion of a molecule is often not considered, although its contribution to the QENS spectra can not be generally neglected.

One of the goals of the present work was to perform the analysis of QENS spectra of aqueous solutions in a way which as complete as possible. The theoretical basis of such an analysis exists since many years, and there are many examples for the detailed analysis of the QENS spectra in the solid state and soft matter fields. Thus, the present work is an attempt to apply both theory and methodology in a consequent and careful manner, with the aim *to learn more* from the QENS spectra of aqueous solutions.

1.4 Studies on aqueous solutions of CDs and mCDs

An analysis of the experimental results often requires many kinds of data. Despite of the large amount of literature on cyclodextrins, the number of studies reporting parameters concerning the aqueous solutions of cyclodextrins and their methylated derivatives is rather

limited. It is instructive to make a short (not exhaustive) account of the studies which are especially relevant with regard to the objectives of this work.

The values of the translational diffusion coefficient measured by different methods are reported for the aqueous solutions of α-CD [39,48,91,98,141], β-CD [39,48,75,91,98,101,138, 141], γ-CD [39,91], γ-CD in 0.5 N NaOH [92], DIMEB [39], TRIMEB [39]. The rotational correlation time of the CD macrocycle is reported for solutions of α-CD [9,59], β-CD [7,94,136, 137], γ-CD [59].

Apparent molar volumes in aqueous solutions of α-CD and β-CD are reported in [96]. Osmotic coefficients, densities and viscosities were measured for aqueous solutions of α- and γ-CD [85]; osmotic coefficients are also reported for aqueous solutions of DIMEB [86].

Ultrasonic absorption studies were done on aqueous solutions of α-, β- and γ-CD [100]; and on the aqueous solutions of DIMEB and TRIMEB [54].

The crystallization of DIMEB and β-CD from aqueous solutions was studied by light scattering [41,142] and by differential scanning calorimetry [96]. A small-angle neutron scattering experiment on β-CD solution in D_2O was reported in [77], but the high concentration given there (weight fraction of β-CD 0.65) suggests that the studied substance was not β-CD, because its solubility in H_2O at 25 °C is only 18.5 g/L, see Tab. 1.1. The presence of aggregates in solutions of α-, β- and γ-CD was observed by static and dynamic light scattering [22], aggregates were also observed in β-CD solutions at concentrations of 10 g/L and higher [39].

1.5 Hydration shell structure and dynamics

The hydration of saccharides and proteins has been studied for several decades by many different methods, e.g.:

- ultrasonic velocity measurements [13,40,111];
- measuring of translational diffusion coefficients of proteins [144];
- viscosity measurements [51];
- dielectric measurements [15,129];
- X-ray and neutron diffraction [33,93];
- small-angle X-ray and neutron scattering [122];
- NMR spectroscopy [46];
- nuclear magnetic relaxation [44,121,129,133,139].

In a relatively recent review on protein hydration [44] the concept of hydration and different methods allowing the investigation of the structure and dynamics of the hydration shell are discussed. In particular, it is stated there that the residence time of the water molecule in the hydration shell "*can not be determined by any known experimental technique*". As a

measure of the dynamic perturbation of hydration water the ratio of rotational diffusion coefficients, D_r, of bulk and hydration water molecules is proposed, such ratio "$D_{r(BULK)}/D_{r(HYD)}$" is called "retardation factor".

Quasielastic neutron scattering technique potentially allows to extract information on the structure of hydration shell, the mean time which the water molecule spent there and the dynamical parameters of the hydration water molecule (including $D_{r(HYD)}$). In the present work an attempt was made to realize this potential in practice.