CHAPTER 1

INTRODUCTION

This chapter gives a panorama of cyclodextrins from the past to the present. The focal points are the physical and chemical properties of both native and methylated cyclodextrins, including their crystal structures, and the objectives of this study.

1.1 Cyclodextrins

1.1.1 Historical Background

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of 6–8 glucose units. They are torus-like macrocycles with intramolecular cavities accommodating a variety of molecules, forming so-called inclusion complex. This property has been revealed since the discovery of CDs from enzymatic degradation of starch by Schardinger in 1903 [145] and afterward deserved great attention from scientists in various disciplines. Inclusion complexation is associated with non-covalent bonds formed when a compound (host molecule) spatially encloses another one (guest molecule), which is of fundamental importance for molecular interactions in "supramolecular chemistry" [4, 107]. CDs can be regarded as one of the most important and potential host because they are seminatural products and commercially available in the market with reasonable prices for both academic and industrial purposes. In addition, their toxic effect can be eliminated by selecting a suitable CD type or derivative or mode of application; consequently, they can be used as ingredients or additives of drugs, foods, and cosmetics [3, 55].

Before going further to know about the CDs in detail, it is worth to have a glimpse on their history which is summarized in Table 1.1.

Table 1.1: The evolution of CDs in three main periods
The first scene of CDs in 1891–1930s, the time of discovery, CDs are obtained from degradation of starch by cyclodextrin glycosyl transferase (CGTase) enzyme, including isolation and purification. The properties of CDs and in particular, the inclusion ability are investigated in the second period, 1930s–1970s. The third period, 1970s onward is the time of large scale production for industrial uses.

Time	Event
1891–1930s	**Structure discovery**
1891	A. Villiers [185]
	– found that the enzymatic degradation of starch yields the "cellulosines" (later proved to be the α- and β-CDs)
1903–1911	F. Schardinger [140, 141, 142]
	– laid down the fundamentals of CD chemistry
1930s–1970s	**Chemical and physical properties studies**
1936–1948	K. Freudenberg *et al.* [49, 50, 51, 53, 54]
	– concluded that the crystalline Schardinger dextrins are built from glucose units and contain only α-(1→4)-glycosidic linkages
	– described the first scheme for isolation of pure products
	– discovered and elucidated the γ-CD
1953	K. Freudenberg, F. Cramer and H. Plieninger [52]
	– published a patent on application of CDs in drug formulations
1954	F. Cramer *et al.* [31]
	– studied the inclusion complex properties of CDs
1957	D. French *et al.* [47]
	– discovered that there are larger CDs
	– published the first fundamental review on CDs
1970s–	**Industrial production and application**
	– industry-scale preparation of CDs and their derivatives [1]
	– widespread utilization in various industries [3, 55, 167]

1.1.2 Fundamentals of CD Chemistry

1.1.2.1 Preparation of CDs

CDs are obtained from enzymatic degradation of starch, a polysaccharide chain consisting of α-(1→4)-linked glucose units arranged into a left-threaded screw with six

glucose units per turn (V-amylose, see the proposed conformation in [23, 60, 131, 139, 184], and the recent X-ray structure of cyclomaltohexaicosaose, CA26 in [59]). The principle is that cyclodextrin glycosyl transferase (CGTase, EC 2.4.1.19) cuts at a turn of starch helix and links the two ends of this fragment giving cyclic molecules which contain 6–12 glucose units per ring. The mechanism can be stated briefly as follows [15]:

$$\text{(i)}\ G_x \underset{\text{coupling}}{\overset{\text{cyclization}}{\rightleftharpoons}} G_{x-n} + G_n$$

$$\text{(ii)}\ G_x + G_y \overset{\text{disproportionation}}{\rightleftharpoons} G_{x-n} + G_{y+n}$$

Starting from the polysaccharide chains G_x and/or G_y with x and/or y glucose units, the cyclization (reaction (i)) and disproportionation (reaction (ii)) occur simultaneously. The cyclization yields the products G_n with n of 6, 7, and 8 glucose units named α-, β-, and γ-CDs, respectively. The efficiency of cyclization depends on the probability of free acceptor binding sites of the CGTase enzyme and decreases with higher substrate concentration at the cost of disproportionation. The rate of cyclization depends on the helical conformation of the substrate. The chains G_{16}–G_{80} yield the maximum rate while chains $< G_{14}$ cannot be cyclized and chains $> G_{100}$ are poor substrates. The reverse coupling reaction (reaction (i)) takes place only at 0.5% of the initial cyclization rate with chains G_{16} at the corresponding initial substrate concentration. In addition, the short linear chains are elongated by the disproportionation until they are long enough for cyclization.

1.1.2.2 Structural Features of CDs

CDs are a family of cyclic oligosaccharides (Figure 1.1) with the glucopyranose unit in $^4C_1$ conformation as building block (Figure 1.2). They have a truncated cone-like macrocycle (Figure 1.3). The α-CD, also called Schradinger's α-dextrin, cyclomaltohexaose, cyclohexaglucan, cyclohexaamylose, and CA6, comprises six glucopyranose units. The β- and γ-CDs comprise seven and eight glucopyranose units, respectively, also known with other names similar as used for the α-CD. CDs with ring size less than α-CD do not exist because of steric effects. CDs are amphiphilic, as the hydrophobic cavity is lined by the C3–H, C5–H hydrogen atoms and ether-like O4, O5, and the hydrophilic rims of cone are lined by the primary O6–H hydroxyl groups on

the narrow side, and the secondary O2–H, O3–H hydroxyl groups on the wide side of the cone. The latter form systematic intramolecular, interglucose O2(n)$\cdots$O3(n – 1) hydrogen bonds which stabilize the CD "round" conformation. The characteristics of α-, β-, and γ-CDs are listed in Table 1.2. Note that β-CD is least soluble in water, only 1.85 g/100 mL while those of α-, and γ-CDs are 14.5 and 23.2 g/100 mL, respectively. This is probably due to the β-CD being more rigid than the others; it prefers to form dimers with the polar hydroxyl groups engaged in intermolecular, interdimer hydrogen bonds as frequently observed in the crystal structures [74]. Furthermore, light scattering showed that the β-CD form rod-like aggregates in aqueous solution [30] consequently, it only slightly dissociates and is less soluble in pure water.

Table 1.2: Characteristics of α-, β-, and γ-CDs
CDs with different number of glucose units have different physical and chemical properties. These properties usually increase with increasing number of glucose unit (ring size) except the solubility in water at RT which is lowest for β-CD. Taken from [167].

CD	α	β	γ
Number of glucose units	6	7	8
Molecular weight	972	1135	1297
Solubility in water at RT (g/100 mL)	14.5	1.85	23.2
$[\alpha]_D^{25}$	150.5±0.5	162.5±0.5	177.4±0.5
Cavity diameter (Å)	4.7–5.3	6.0–6.5	7.5–8.3
Torus height (Å)	7.9±0.1	7.9±0.1	7.9±0.1
Peripheral diameter (Å)	14.6±0.4	15.4±0.4	17.5±0.4
Cavity volume (Å^3)	174	262	427

• **New Insight into the Molecular Hydrophobicity of CDs.** F. W. Lichtenthaler and S. Immel have published a number of papers on the topic of *Molecular Modelling of Saccharides* which provides a more comprehensive assessment of geometries and lipophilic characteristics of CDs and other cyclic sugars. The molecular lipophilicity patterns (MLPs) of CDs [108, 109] were illustrated by color-coded visualization produced by the program MOLCAD [19, 20, 21, 176]. The MLPs are projected onto the corresponding molecular contact surfaces as depicted in Figure 1.4

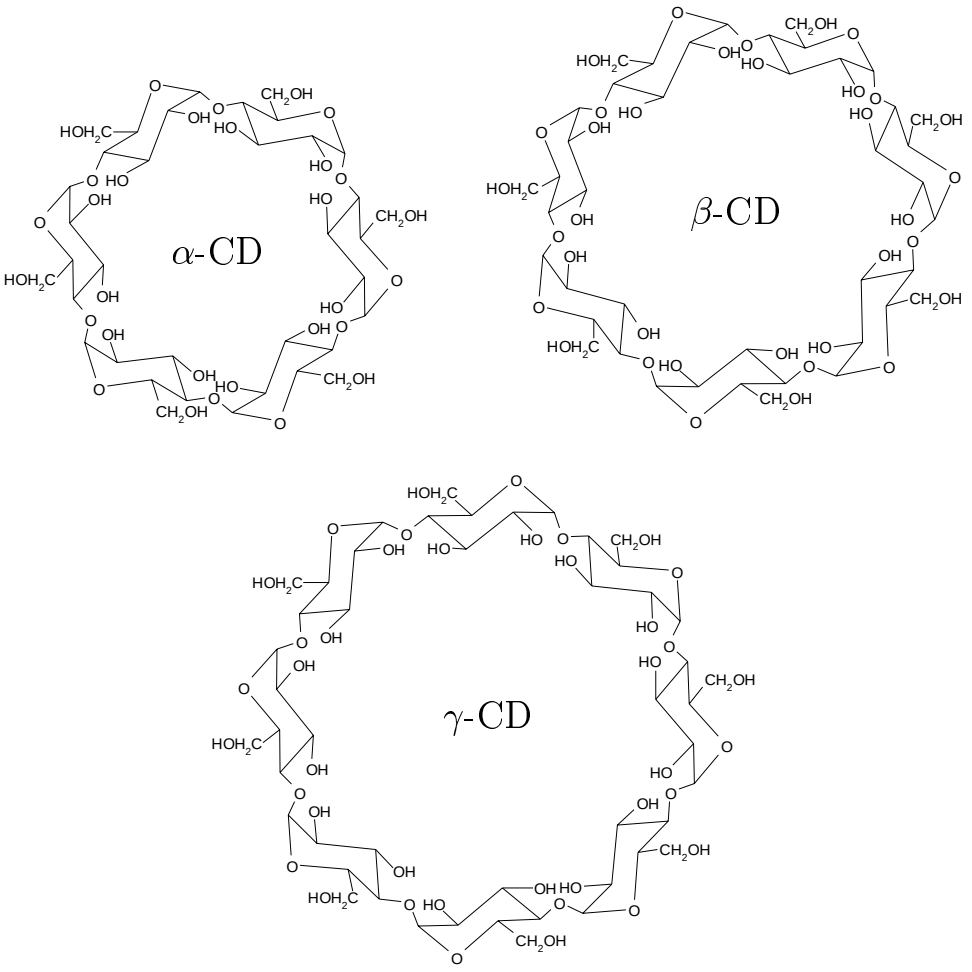

Figure 1.1: Chemical structures of CDs
α-, β-, γ-CDs with 6, 7, 8 D-glucose units linked by α-(1→4) glycosidic bonds (upper left, right, lower). Numbering of glucose units is counterclockwise and for atomic numbering, see Figure 1.3.

in a two-color-code graded into 32 shades, ranging from dark blue for the most hydrophilic areas to yellow for the most hydrophobic regions. The MLPs of the α-, β-, and γ-CDs show that the O2–H/O3–H side of the macrocycle (wide rim of torus) is distinctly hydrophobic as evidenced by the blue regions (left entries). This contrasts to the intensively hydrophobic (yellow) surface areas (left entries) on the opposite side – the O6–H side of the macrocycle (narrow rim of torus). Out of the calculated total surface area of $\approx$ 120 Å^2 per glucose unit (α: 720, β: 845, γ: 960 Å^2), only *ca.* 10–15% contributes to the inner surface of the central cavity (α: 85, β: 105, γ: 140 Å^2). This is clearly illustrated by the side view MLPs in bisected form (right entries,

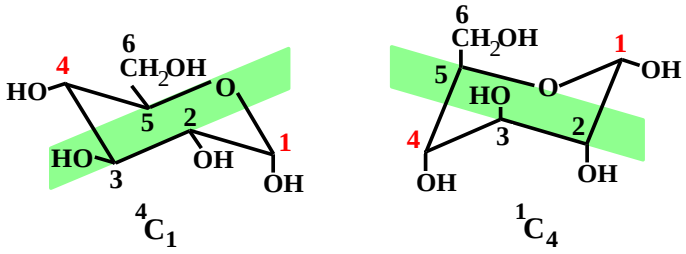

Figure 1.2: Chair conformations of a glucose unit

Two possible chair conformations of glucose. The green planes passing through C2, C3, C5 and O5 guide the eye. When C4 is above and C1 is below this plane, the glucose conformation is called $^4C_1$ (left), and on the contrary for $^1C_4$ (right). The $^4C_1$ form is generally found in the CD structures because it is energatically more stable due to fewer repulsive interactions between its O–H groups which are almost in equatorial positions.

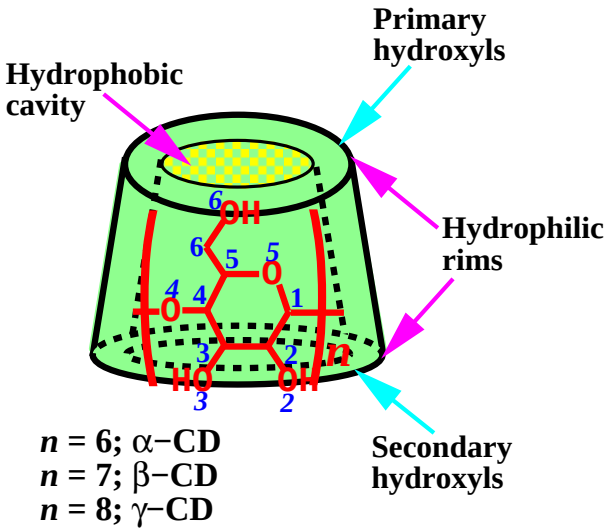

Figure 1.3: Common structural feature of CDs

Truncated cone shape with the *hydrophobic* central caviy coated by C–H groups and ether-like O4, O5, and the *hydrophilic* rims at the narrower side lined by the primary O6–H hydroxyl groups and at the wider side lined by the secondary O2–H, O3–H hydroxyl groups. Atomic numbering of glucose gives the number according to the chemical name, normal ones for carbon atoms and italics oxygen atoms.

Figure 1.4). Most interestingly, *not the entire cavity surface areas are hydrophobic*, but only those regions in the narrower half of the cone structures.

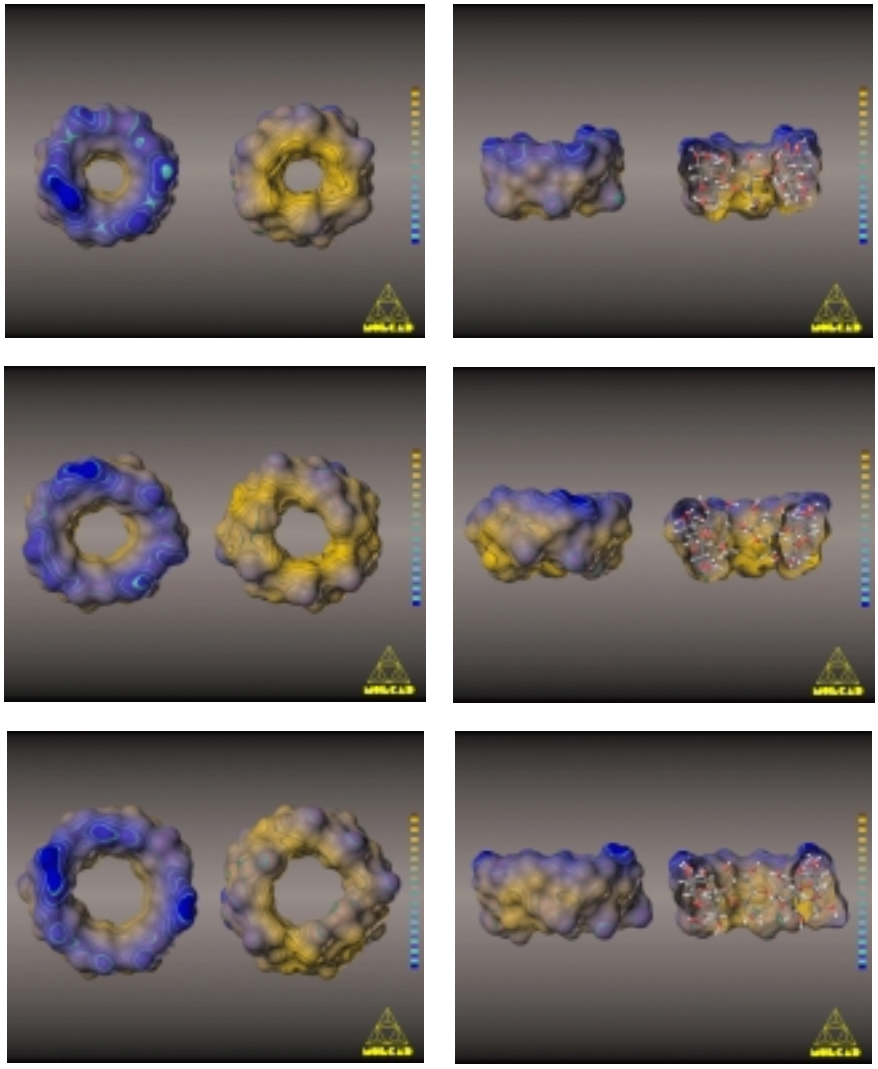

Figure 1.4: Molecular lipophilicity patterns of CDs
MOLCAD-program [19, 20, 21, 176] generated molecular lipophilicity patterns (MLPs) of α-CD (top), β-CD (center), γ-CD (bottom) [108, 109]. For visualization a two-color-code graded into 32 shades is used. The first 16 colors indicating the range of relative hydrophobicity from dark blue (most hydrophilic surface areas) over light blue to full yellow (most hydrophobic regions). The remaining 16 color shades (light blue to brown) indicate the iso-contour lines in between former color scale; this gives a more quantitative assessment of relative hydrophobicity on different surface areas. The left pictures (left entries) are viewed through the O2–H/O3–H side (larger aperture) of the cone shaped molecule showing the intensively hydrophilic (blue) surface areas, whereas the opposite view (right entries) to the O6–H side (smaller aperture), the hydrophobic regions are indicated by yellow. In the side views of MLPs on the right, individual molecules are shown in closed and bisected forms. They are orientated with O2–H/O3–H side upward and O6–H downward. The inserted ball-and-stick model illustrates the molecular orientation. The similarities in the distribution of hydrophilic (blue) and hydrophobic (yellow) surface areas – most notably on the inside regions of the cavities of α-, β-, γ-CDs are apparent.

• **Geometrical Parameters Describing the CD Macrocycles.**

Glucose puckering or Cremer-Pople puckering parameters [32] (see Figure 1.5) describe the conformation of glucose by the total puckering amplitude, Q, and degree of deviation from the regular $^4C_1$ chair conformation, Θ. Their theoretical values refer to the conformation of cyclohexane which has Q of 0.63 Å and Θ of 0° [32]. So far in all CDs characterized crystallographically, the individual glucose units adopt the normal $^4C_1$ chair form which is fairly rigid as indicated by the comparable puckering parameters, except for trimethylated β-CD in which one glucose unit adopts the $^1C_4$ conformation [25] due to steric effects (more detail in section of methylated CDs).

O6–H group rotation about the C5–C6 bond as found in CD structures can be devided into two forms (Figure 1.6). The *–gauche* form with O6–H directed "away" from the molecular cavity is largely preferred. The *+gauche* form with O6–H "toward" the cavity only occurrs in crystal structures when certain packing requirements are met or when hydrogen bonds are formed with an included guest molecule.

Torsion angles, ϕ and ψ [2] explain the orientation of adjacent glucose units about the glycosidic bonds in the CD macrocycle. They are defined as angles of O5(n)–C1(n)–O4($n-1$)–C4($n-1$) for ϕ, and C1(n)–O4($n-1$)–C4($n-1$)–C3($n-1$) for ψ (Figure 1.7(a)). Two orientations of neighboring glucose residues are observed in the CD structures. In *syn* orientation (Figure 1.7(b)), all O2–H, O3–H groups are on the same side of the CD macrocycle. They form intramolecular, interglucose O2(n)$\cdots$O3($n-1$) hydrogen bonds which stabilize the "round" macrocyclic conformation. This is commonly found in the CDs with 6–8 glucose units. By contrast, the *anti* orientation (Figure 1.7(b)) is observed in larger CDs, e.g., ϵ-CD or CA10 [88, 89, 179], ι-CD or CA14 [75, 88, 89] and ϕ-CD or CA26 [59]. These molecules are not "round" anymore but adopt an elliptical or helical shape and are further stabilized by O3(n)$\cdots$O5($n+1$) and O3(n)$\cdots$O6($n+1$) hydrogen bonds.

Tilt angle shows the inclination of individual glucoses to the CD cavity. It is defined as the angle between the O4(n)–C4(n)–C1(n)–O4($n-1$) plane and the mean

plane of glycosidic O4, see Figure 1.8. The known CD structures show that tilt angles fluctuate in a wide range up to 10°; this is because each glucose unit can rotate freely within the accessible space around the glycosidic O4. However, to maintain the "round" shape of CD macrocycle, the glucose units are allowed to rotate in a restricted range so that the systematic interglucose O2(n)···O3($n-1$) hydrogen bonds stabilizing the molecule are satisfied. Larger values of tilt angles are found when the glucose units are strongly inclined to form hydrogen bonds with included guest molecules.

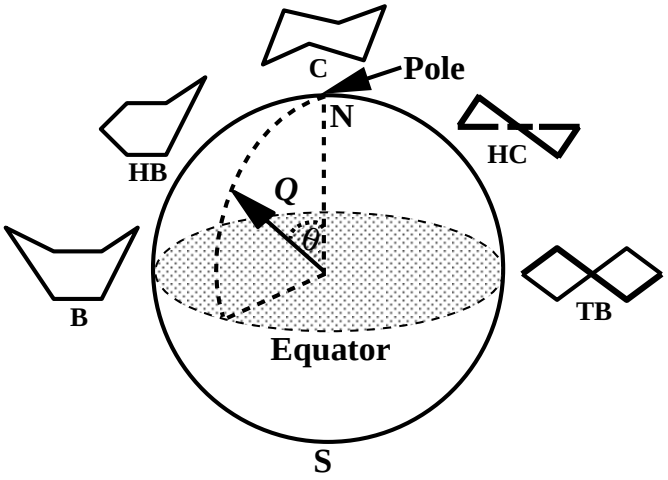

Figure 1.5: Glucose puckering parameters on the conformational sphere
Cremer-Pople puckering parameters [32] explain the feasible conformations of glucose with two values: total puckering amplitude, Q, is the radius of the sphere and polar angle, Θ, indicates degree of deviation from $^4C_1$ chair conformation at the north pole (**N**). Other conformations, e.g., HB (half boat), HC (half chair), B (boat), and TB (twist boat) are located at the equator. Actually, the $^1C_4$ chair form is at the south pole (**S**). The theoretical values of Q and Θ are 0.63 Å and 0° [32] for pure $^4C_1$ chair conformation in cyclohexane.

Parameters concerning glycosidic O4 describe a well-defined CD macrocycle as the following:

- **deviations of O4 from the common O4 mean plane** less than 0.25 Å show that the O4 atoms defining the macrocyclic polygons are coplanar,
- **O4($n+1$)···O4(n)···O4($n-1$) angles** are close to the ideal values for corresponding polygons; 120°, 128° and 135° for α-, β- and γ-CDs, respectively,

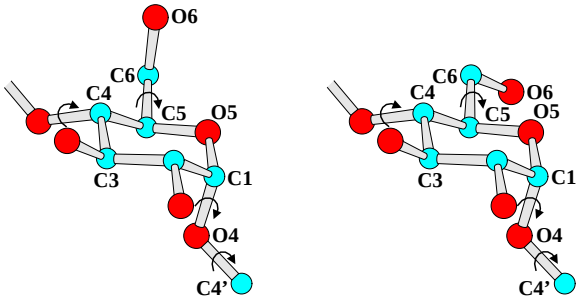

Figure 1.6: Orientation of O6–H group
Glucose $^4C_1$ chair conformation with O6–H in the *–gauche* (left) and *+gauche* (right) orientations. Bonds about which rotations are likely to occur in CDs are indicated by arrows [133].

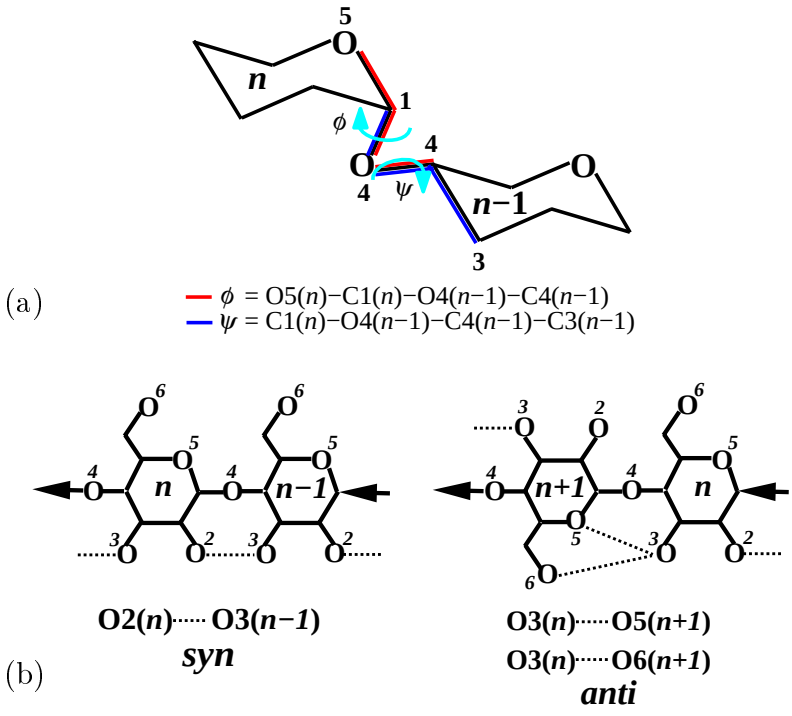

Figure 1.7: Torsion angles ϕ, ψ and orientations of glucoses in *syn*, *anti*
(a) Rotation about the glycosidic O4 (cyan arrows) described by torsion angles, ϕ (red line) and ψ (blue line). (b) Two orientations of adjacent glucose units in CD structures stabilized by different donor-acceptor pairs of hydrogen bond: O2(n)···O3($n-1$) for *syn* (left), and O3(n)···O5($n+1$), O3(n)···O6($n+1$) for *anti* (right). Black arrows indicate the connection to neighboring glucose residues.

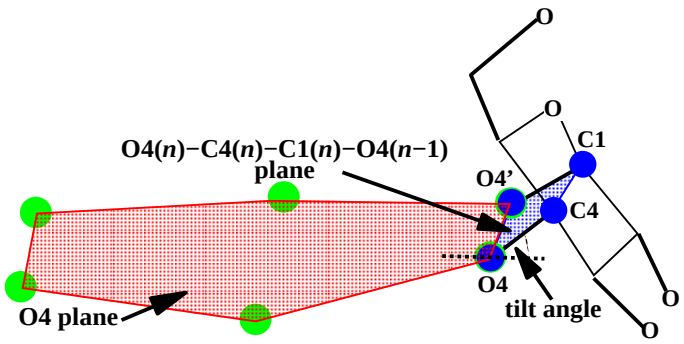

Figure 1.8: Tilt angle
Tilt angle defined as angle between the plane of O4(n)–C4(n)–C1(n)–O4(n – 1) (blue area) and the plane of glycosidic O4 (red region) showing the inclination of individual glucose units to the CD cavity.

– **O4(n)···O4(n – 1) distances** forming the edges of macrocycles are invariable within each member of the CD family; they increase from α- to γ-CDs according to the increase of respective raduis of the CDs.

• **Crystal Structures of CD Hydrates.** CDs are soluble in water and crystallize with water molecules are included in the CD cavities as well as in the interstices between CDs. Many X-ray and neutron structures of uncomplexed hydrated CD have been determined as summarized in Table 1.3.

Table 1.3: Native CDs crystallize in water as hydrated forms

Crystal structures of native CDs determined by X-ray and neutron diffractions: 4 forms for α-CD, 2 for β-CD and 3 for γ-CD. Different numbers of water molecules occupy the CD cavity and the intermolecular space.

Crystal form	No. of water inside/outside CD cavity	Macrocyclic Conformation
α-CD·6H_2O (form I) [118, 119]	2/4	distorted round
α-CD·6D_2O (form I, RT, neutron) [98]	2/4	distorted round
α-CD·6H_2O (form II) [114]	1/5	round
α-CD·7.57H_2O (form III) [27]	2.57/5	round
α-CD·11H_2O (form IV) [130]	7/4	round
β-CD·12H_2O (form I) [110, 112]	6.5/5.5	round
β-CD·11H_2O (form II) [58]	6.3/4.7	round
β-CD·11D_2O (form II, 293 K, neutron) [17]	6.1/4.9	round
β-CD·11D_2O (form II, 120 K, neutron) [186]	6.6/4.4	round
γ-CD·17H_2O (form I, 120 K) [117]	12/5	distorted round
γ-CD·14.1H_2O (form II) [63, 66]	7.1/7	round
γ-CD·15.7D_2O (form III, 110 K, neutron) [36]	8.8/6.9	round

X-ray structures. The α-CD crystallizes in four forms. In α-CD·6H_2O, form I [118, 119], two water molecules are included in the cavity. This causes a distortion of the macrocycle, two O2(n)$\cdots$O3(n–1) hydrogen bonds being broken because one rotated glucose moves O6–H into the central cavity to hydrogen bond with one of the included water molecules. By contrast, forms II [114], III [27], and IV [130] contain 6, 7.57, and 12 water molecules, respectively, in the asymmetric unit and feature "round" macrocycles. The CD cavities accomondate 1 water molecules in form II, 2.57 and 7 in forms III and IV. These macrocycles are nearly isomorphous in "round" shapes and are stabilized by interglucose,

intramolecular O2(n)$\cdots$O3($n-1$) hydrogen bonds with comparable O$\cdots$O distances of 2.90–3.15 Å, Table 1.4 and Figure 1.9(a). In case of β-CD, the crystals are grown in two forms, β-CD·12H_2O, form I [110, 112] and β-CD·11H_2O, form II [58]. They are nearly isomorphous in "round" conformations and differ only in the distribution of water molecules; 6.5 and 6.3 water molecules occupy the cavities for forms I and II, respectively (Table 1.4 and Figure 1.9(b)). The γ-CD hosts more water molecules because of the larger cavity size. In γ-CD·17H_2O, form I [117], the molecule is in a distorted "round" shape and accomodates 12 water molecules in its cavity. In γ-CD·14.1H_2O, form II [63, 66]; the molecular structure is similar to the first form and 7.1 water molecules are embeded in the cavity (Table 1.4 and Figure 1.9(c)). The β- and γ-CDs macrocycles are more symmetrical and rigid than that of α-CD as indicated by the stronger O2(n)$\cdots$O3($n-1$) hydrogen bonds with shorter O$\cdots$O distances which are in the ranges of 2.77–2.96 and 2.77–2.91 Å, respectively (Table 1.4).

Table 1.4: Structural parameters of the CD hydrates

CDs crystallized from water as different hydrated forms with comparable structural parameters. Given angles in ° and distances in Å. [a] In α-CD·7.57H_2O [27]. [b] In β-CD·12H_2O [110, 112]. [c] In γ-CD·14.1H_2O [63, 66]. [d] Definitions on page 8. [e] Deviation of O4 atoms from their least-squares plane, see page 9.

CD	α^a	β^b	γ^c
Angles			
ϕ^d	102.0–114.9	102.5–120.1	103.6–123.2
ψ^d	115.1–148.7	115.3–138.2	111.9–138.3
Tilt angle[e]	2.5–28.6	6.4–26.2	1.5–23.8
O4($n+1$)$\cdots$O4(n)$\cdots$O4($n-1$)	116.9–122.3	123.8–134.4	133.5–136.9
Distances			
O2(n)$\cdots$O3($n-1$)	2.90–3.15	2.77–2.96	2.77–2.91
O4(n)$\cdots$O4($n-1$)	4.16–4.30	4.24–4.50	4.43–4.59
O4 deviation[e]	–0.15 to 0.13	–0.22 to 0.28	–0.19 to 0.18

Neutron structures. Neutron diffraction is a powerful tool for the precise and reliable determination of hydrogen atoms involved in hydrogen bond networks

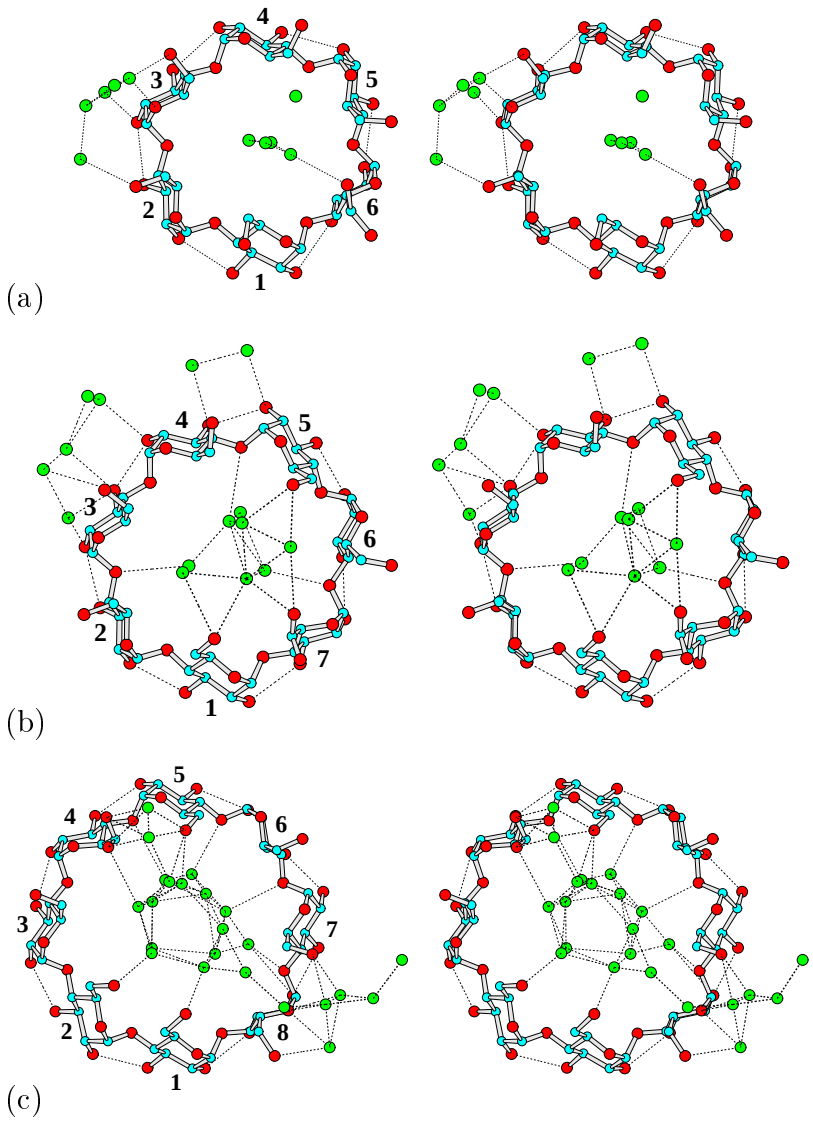

Figure 1.9: Stereo plots of native CD hydrates
(a) α-CD·7.57H_2O [27], (b) β-CD·12H_2O [110, 112], and (c) γ-CD·14.1H_2O [63, 66]. Cyan, red, and green spheres are CD carbon, oxygen, and water oxygen atoms, respectively. Dotted lines indicate possible O···O hydrogen bonds within 3.5 Å distance.

formed by CD hydroxyl groups and water molecules. This cannot be reliably obtained by X-ray diffraction because of the low scattering power of hydrogen atoms. Because the incoherent neutron scattering of hydrogen atoms is very high compared to those of others; this leads to difficulties in structure refinement. Therefore, to reduce incoherent background from hydrogen atoms, CDs

have been crystallized in heavy water (D_2O) so that all exchangeable (hydroxyl) protons of CDs can be substituted by deuterium. So far four structures of uncomplexed hydrated native CDs have been reported. The α-CD·6D_2O, Form I was determined at RT [98]. All hydrogen atoms involved in hydrogen bonding could be located. The O–H···O hydrogen bond networks showed cooperative effect [134] with O–H hydrogen atoms organized in infinite chains and circular structures with four, five, and six-membered rings predominating, where all O–H groups point in the same direction, O–H···O–H···O–H···, called *homodromic* [134]. There are two other possible arrangements of O–H groups: *antidromic* with one water molecule donating two hydrogen bonds and *heterodromic* with all O–H groups in random arrangement. The latter was never found so far because it is less stable than the others. The β-CD·11D_2O, Form II was determined at two different temperatures, at 293 K [17] and at 120 K [186]. The RT structure showed disorder of hydrogen atoms of secondary O2–H, O3–H hydroxyl groups and of water molecules. The intramolecular, interglucose O2(n)···O3($n-1$) hydrogen bonds, in fact, are in dynamical equilibrium: H–O2(n)···H–O3($n-1$) $\rightleftharpoons$ O2(n)–H···O3($n-1$)–H [17, 137] which are called "flip-flop" hydrogen bonds. This dynamical behavior was confirmed by the neutron structure of the same crystal form at 120 K [186] where the "flip-flop" disorder of O–H groups became ordered, and calorimetric studies have indicated that a reversible disorder-order phase transition occurs at 227 K [58]. In addition, quasielastic neutron scattering experiments [161, 162] and molecular dynamics simulation [101] have shown that the disorder has jump rates up to 2×10^{11} s^{-1} at RT. The γ-CD·15.7D_2O, Form III [36] was determined at 110 K; 8.8 water molecules are included in the cavity and not all of the hydrogen atoms could be located. The homodromic arrangements dominate in the O–H···O hydrogen bond networks and show coorperative effect as observed in α-CD·6D_2O [98] and β-CD·11D_2O [186].

• **Larger CDs.** The first evidence for the existence of larger CDs composed of more than eight glucose units had been given by D. French in 1965 [48]. Because of difficulties in the isolation and purification, they could not be crystallized and characterized. Just only late nineties they were unequivocally defined: δ-CD [57],

ϵ-CD [38, 88, 89, 179], ζ-CD [38], η-CD [40], θ-CD [38], ι-CD [39, 75, 88, 89], κ-CD [39], λ-CD [39], μ-CD [39], ν-CD [180], ξ-CD [180], and ϕ-CD [59] with 9–19 and 26 glucose units, respectively. Furthermore, the CDs with degree of polymerization up to 31 had been isolated and characterized [102]. For CDs with more than 100 glucose units and beyond have been prepared by disproportionation with potato D-enzyme [171] as well as with CGTase enzyme [175]. Of all the mentioned larger CDs only the structures of δ-CD [57], ϵ-CD [88, 89, 179], ι-CD [75, 88, 89], and ϕ-CD [59] were determined by X-ray analysis. The others were characterized by NMR and mass spectrometry. In sharp contrast to the "round" conformations of smaller CDs, the larger CDs have "elliptical" or "helical" shapes. δ-CD crystallizes with 13.75 water molecules [57] which are all located in the interstices between CD molecules. The CD cavity is not empty but filled by two glucose residues of a neighboring CD molecule. The macrocycle exhibits a "bowl" shape and four glucose units are strongly tilted to the central cavity. ϵ-CD crystallizes in two hydrated forms with slight differences in water content in the crystal lattices: $19H_2O$ (form I) [179] and $23.5H_2O$ (form II) [88, 89]. Their macrocycles are "elliptical", U-like shapes which are very different from those of α- to δ-CDs. This is due to $\approx 180°$ flipping of two diametrically opposed glucose units resulting in the disruption of circular interglucose $O2(n)\cdots O3(n-1)$ hydrogen bonds which still exist in δ-CD. At the flip site, two adjacent glucose units are orientated *anti*, the other glucoses still remaining *syn*. This is a new structural motif called "band-flip" [88, 89], analogous to a band which is cut in two halves, one of the end residues is rotated 180°, and the two halves are glued back together. ι-CD also crystallizes in two forms: $29.7H_2O$ (Form I) [88, 89] and $9H_2O$ (Form II) [75]. ϕ-CD crystallizes as $(\phi\text{-CD})_2{\cdot}76.75H_2O$ [59]. The macrocycle adopts the shape of a figure eight in which each half consists of two left-handed, single helical turns with six glucose units per repeat, reminiscent V-amylose. The turns are stabilized by hydrogen bonds formed internally between the neighboring glucose units by $O2(n)\cdots O3(n-1)$, and between the turn by $O2(n)\cdots O6(n-6)$, and $O3(n)\cdots O6(n-6)$. The two short single helices in ϕ-CD contain channel-like cavities with a similar width as observed in α-CD and host water molecules.

1.1.2.3 CD Inclusion Complexes

Inclusion complexes are molecular compounds in which one of the compound (host

molecule) spatially encloses totally or partially another one (guest molecule) only by non-covalent bonding.

CDs are able to form inclusion compounds with a great variety of ionic or molecular species which apparently only have to satisfy a steric requirement – they must fit entirely or at least partially into the CD cavity. In an aqueous CD solution, the apolar CD cavity is filled by water molecules which are energetically unfavored (polar-apolar interaction), and therefore can be easily replaced by suitable guest molecules which are less polar than water. The CD inclusion compounds can be characterized in both liquid and solid states [135, 165]. Among the methods used to detect inclusion complex formation in liquid state are: UV-visible spectrophotometry, Circular dichroism, NMR, and capillary electrophoresis. The NMR technique is more convenient, rapid, and precise therefore, it is widely applied especially, to study the dynamical properties in solutions. (for recent review, see [144]). In case of crystalline inclusion complexes, X-ray powder diffraction, spectroscopic techniques, e.g., IR or ESR, TLC, thermal analysis, mass spectrometry and single crystal X-ray analysis can be used. Although the X-ray analysis is a time-consuming and complicated method, the precise geometrical relationships between the guest and host molecules can be established and interactions can be indentified.

CDs crystallizing either as hydrates or as inclusion complexes, can be categorized in two types according to the overall appearance of the formed cavities, Figures 1.10(a)–(d) [136]. In the channel type, CDs are stacked on top of each other like coins in a roll leading to endless channels (Figures 1.10(a), (b)) where the guest molecules are included. This packing is stabilized by hydrogen bonding between O2–H/O3–H and O6–H sides for head-to-tail style (Figure 1.10(a)) or between O2–H/O3–H and O2–H/O3–H on one side and between O6–H and O6–H on the other side for head-to-head mode (Figure 1.10(b)). In the cage type (Figures 1.10(c), (d)), both sides of the CD cavity are blocked by adjacent CDs forming isolated cavities in which the included guest molecules are not in contact with each other. Two different structures of cage type are observed. In one, CDs are packed crosswise in herringbone fashion (Figure 1.10(c)) and in the other one, they are arranged in layers, and neighboring layers are laterally shifted by a half of molecular length like bricks in a wall (Figure 1.10(d)). Whereas CDs are only arranged in cage type for uncomplexed hydrated crystals, they prefer different kinds of arrangement for inclusion complexes

(for more detail see a complete list of CD hydrates and their inclusion complexes determined by X-ray and neutron diffraction in [74]).

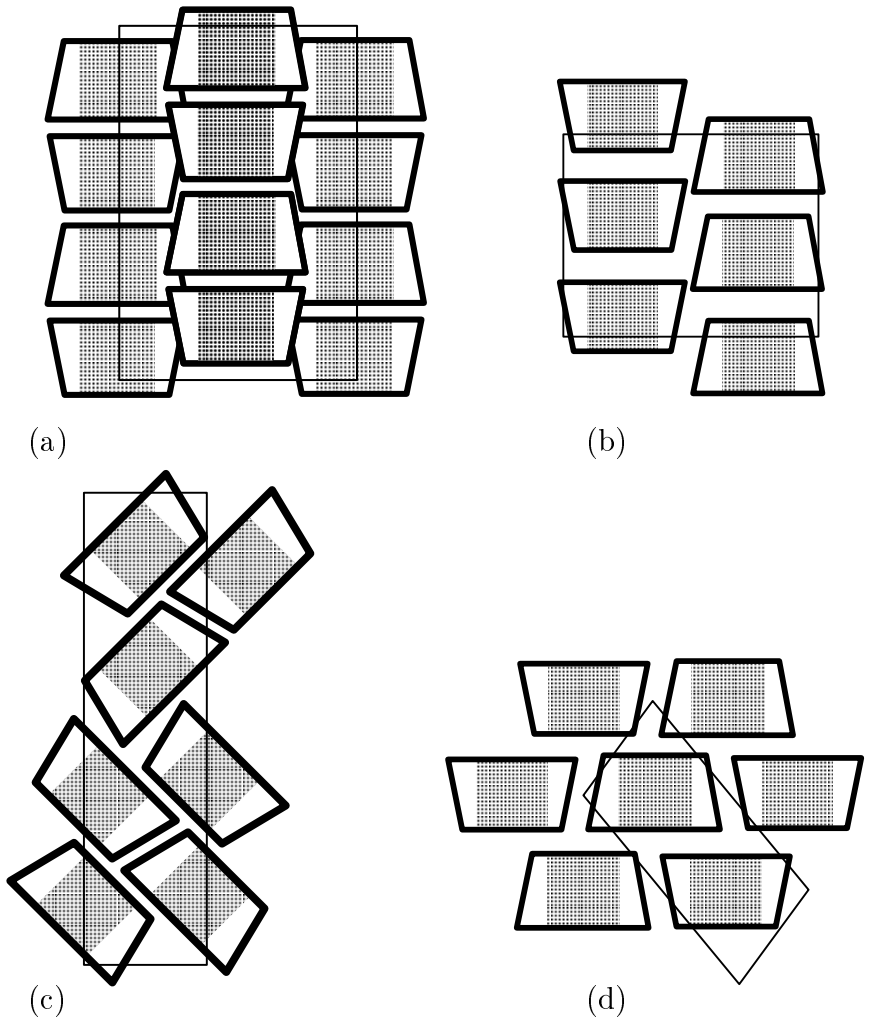

Figure 1.10: Crystal packing of native CDs
Two main types of crystal packing are observed according to the formed cavities – channel and cage. In channel mode, the CD molecules are stacked on top of each other like a roll of coins forming infinite channels which can be sub-divided into (a) head-to-head, and (b) head-to-tail. In cage mode, the CD molecules may stack (c) crosswise or arrange (d) in layers forming isolated cavities.

– **α-CD inclusion complexes** form channels when the guests are long or ionic whereas small guests form cages. An obvious example for well defined size selectivity [120] are inclusion complexes of α-CD with carboxylic acids [120]: complexes with acetic, propionic, and butyric acids crystallize in cages while

valeric acid and higher analogues form channels. In addition, most of channels formed by α-CD are head-to-tail pattern and only in some complexes, e.g., with polyiodides [126, 127], and organometallic compounds [29, 99, 100], they crystallize in head-to-head structures.

– **β-CD inclusion complexes** prefer head-to-head channels. For some cases, e.g., the dodecahydrate [110, 112], methanol [113], ethanol [178], hydrogen iodide [113], potassium hydroxide [28] complexes of β-CD crystallize as cages.

– **γ-CD inclusion complexes** are also dominated by molecular packing of channels but arranged alternatively between head-to-head and head-to-tail, e.g., with 1-propanol [35, 111], crown-ether [94, 95] and methanol [159]. Only for γ-CD·15.7D_2O [36], the γ-CDs are arranged in herringbone cage-type.

– **Inclusion complexes of larger CDs** so far are described only in a few papers. Because the molecular cavities of large CDs are not well defined, they are weak complex formers when compared to the small CDs. The δ-CD has much narrower cavity and reduced ability to complex with guest molecules [123, 121, 124]. Other examples are crystallizations of ϵ-CD in a mixture of 50:50 (v/v) acetonitrile–water [179], and ι-CD in a mixture of 70:30 (v/v) 1-propanol–water [75]. In both cases, they crystallize as pure hydrates, without inclusion of the organic solvents. By contrast, the α- to γ-CDs are able to form inclusion complex as previously reported (with 1-propanol [35, 93, 111, 138, 163], and with acetonitrile [10]). A capillary electrophoretic study on the inclusion complex of α- to θ-CDs (6–13 glucose units) with various anions [121] has shown that the complexing ability of ϵ-, ζ-, η-, and θ-CDs increases with increasing ring size; the complex formation constants of γ- and θ-CDs are comparable, and δ- and ϵ-CDs are the weakest complex formers. An isothermal titration calorimetric study on the inclusion complex of large CDs with 21–32 glucose units [97], has revealed that there are two identical binding sites in these macrocycles indicating 2:1 complex formation, with two different binding constants. The obtained thermodynamic parameters suggest that ϕ-CD or CA26 is less flexible than the others; this is probably due to the folding in two single helices as evidenced by the crystal structure [59]. The binding constants of complex ϕ-CD–iodine is 10–50 times less than that of α-CD–iodine.

1.2 Methylated CDs

Methylated CDs are well known and deserve attention among the derivative of CDs due to their peculiar solubility properties in water [166, 183]. The solubility of β-CD in water increases when up to two thirds of all hydroxyl groups are methylated, then decreases again if all hydroxyl groups are methylated. If 14 methoxyl groups are methylated, β-CD shows highest solubility whereas permethylated β-CD with 21 methyl groups has lower solubility in water which is, however, considerably higher than that of native β-CD (see Table 1.5). Little information is available for the methylated α-, and γ-CDs because their native forms have good solubility (Table 1.5) therefore, there is no need to improve their solubility via methylation.

Figure 1.11 shows a methylated glucose as repeating unit of methylated CDs. Methylation at three different CD hydroxyl groups yields six systematic compounds: dimethyl- and trimethyl-α-, β-, γ-CDs. Hereinafter, abbreviations are used for these compounds, e.g., DIMEA denotes hexakis(2,6-di-*O*-methyl)-α-CD or dimethyl-α-CD, TRIMEG stands for octakis(2,3,6-tri-*O*-methyl)-γ-CD or trimethyl-γ-CD and, so on.

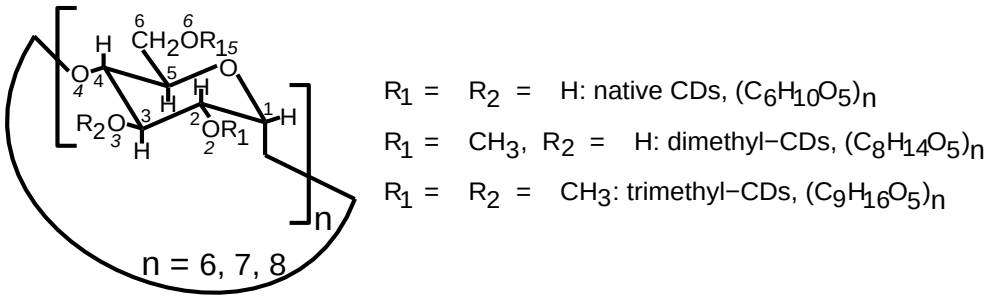

Figure 1.11: Chemical structure of methylated CDs
Six systematic compounds for dimethyl- and trimethyl-CDs. Atomic numbering as for native CDs; for dimethyl-CDs, methyl groups at O2 and O6 are labeled C7 and C8; for trimethyl-CDs, methyl groups at O2, O3, and O6 are labeled C7, C8, and C9. Normal numbers indicate carbon atoms and oxygen atoms italics.

1.2.1 Preparation

The methylation of CDs was first described in 1924 by Irvine *et al.* [87]. Precise characterization of the reaction products was reported in 1938 by Freudenberg [53] using CH_3I as reagent in liquid ammonia in presence of an alkali metal. Here the

Table 1.5: Physical and chemical properties of methylated and native CDs
Note that methylation results in drastic increase of solubility in water, e.g., at RT DIMEB is about 30 times more soluble than β-CD. For units, [a] solubility: g/H_2O 100 mL; [b] surface tension: mN/m, at concentration 0.1 mol/L; [c] ring opening half-life: h, in 1 N HCl at 60°C. Taken from [183].

Molecule	Molecular weight	Melting point (°C)	Solubility at 25°C[a]	$[\alpha]_D^{25}$	Surface tension[b]	Half-life of ring opening[c]
α-CD	973	275	15	151	71	6.2
DIMEA	1141	260–264	–	151	65	12.6
TRIMEA	1225	205	20	153	54	3.0
β-CD	1135	280	1.85	163	71	5.4
DIMEB	1331	295–300	57	160	62	8.5
TRIMEB	1430	157	31	158	56	1.7
γ-CD	1297	275	23	177	71	3.0
DIMEG	1521	255–260	–	184	60	4.0
TRIMEG	1634	135	48	169	56	1.2

syntheses of CDs methylated at O2–H, O6–H, and O2–H, O3–H, O6–H for dimethyl- and trimethyl-CDs, respectively, are mentioned briefly.

1.2.1.1 Trimethylated Derivatives

The preparation of these compounds requires strong methylating agents [115] because of the steric hindrance at the secondary side of the CD macrocycle. However, they can be prepared with high yield up to 98% by methods of Brimacombe [22], Bergeron *et al.* [16], Boger *et al.* [18] or Szejtli *et al.* [170] with slightly different conditions, using CH_3I with NaH in a polar solvent, DMF or DMSO.

1.2.1.2 Dimethylated Derivatives

The synthesis of these compounds is more subtle because of the requirement in selective methylation at O2–H and O6–H. In case of heptakis(2,6-di-*O*-methyl)-β-CD (DIMEB), the absolutely pure product (with 14 methyl groups) is difficult to obtain because CDs with 13 or 15 mehyl groups have similar physical and chemical properties. To separate them, careful chromatographic techniques (HPLC) are required.

By using Kuhn's method [104], modified by Casu [26], Szejtli *et al.* [169]. DIMEB with 78% yield can be prepared by adding $(CH_3)_2SO_4$ to a solution of β-CD in a DMSO/DMF mixture containing BaO and $Ba(OH)_2{\cdot}8H_2O$, at temperature below 20°C. By controlling the reaction temperature of β-CD with CH_3I in DMF in presence of NaOH, DIMEB can be obtained on industrial scale [115]. According to Fügedi [56], DIMEB with about 60% yield can be prepared by using DMF or DMSO solution and alkylhalide or alkylsulphate in presence of powdered KOH at room temperature.

To generalize the syntheses of methylated CDs, there are three main substances used in the reaction: (i) methylating agents ($(CH_3)_2SO_4$, CH_3I), (ii) organic solvents (DMF, DMSO, or DMF/DMSO mixture), and (iii) bases (alkali hydroxide, powered NaOH, KOH, $BaO/Ba(OH)_2$).

1.2.2 Physical and Chemical Properties

Methylation changes the physical and chemical properties of CDs. In terms of physical property, the molecular structure changes as the cavity at the O6-side becomes narrower because neighboring glucose units require more space and tend to tilt with their O6-side toward the molecular cavity to reduce the steric hindrance of methoxyl groups at the O2-, O3-side. The attached methyl groups at both sides of the cavity increase the cone height by *ca.* 3 Å. For dimethylated derivatives in which the intramolecular, interglucose O3–H$\cdots$O2 hydrogen bonds still exist the molecular shapes remain "round". By contrast, trimethylated CDs show distorted "round" or "elliptical" shapes (further detail discussed below) due to an absence of interglucose hydrogen bonds. In terms of chemical property, the hydrophobicity is increased and dominates in these compounds; in principle they should be less soluble in water, but this is not the case as their water solubility increase considerably compared with unsubstituted CDs (Table 1.5). Furthermore, they are also less hydroscopic than their parents, and are highly surface active (Table 1.5). The less hydroscopic property of methylalted CDs is an advantage when they are used as pharmaceutical carriers, because they can protect hydrolytic decomposition of enclosed drugs both in liquid and solid states [168]. The ring-opening of methylayted CDs by acid hydrolysis is different from that of parent CDs due to the steric hindrance of the methyl groups. TRIMEG shows highest susceptibility in acid hydrolysis (Table 1.5) which is probably due to the strong distortion of the macrocyclic conformation.

Figure 1.12 shows the MLPs (see definition on page 4) of permethylated α-, and β-CDs [86] which are inverse to those of native CDs (see Figure 1.4 on page 7). The areas of O–CH_3 groups on both sides of the torus are most hydrophobic while the inner surfaces are most hydrophilic. The calculated cavity areas are increased by 40% and 70% for permethylated α-CD (120 Å^2) and β-CD (180 Å^2), respectively, due to an increase of macrocycle heights from 8.0 Å to 11.1 Å. Because the color-code used in the calculation of MLPs of molecules in Figures 1.4 and 1.12 are treated separately, direct quantitative comparison between their MLPs is not feasible. However, it is clear that the permethyl derivatives are much more hydrophobic than the native analogues.

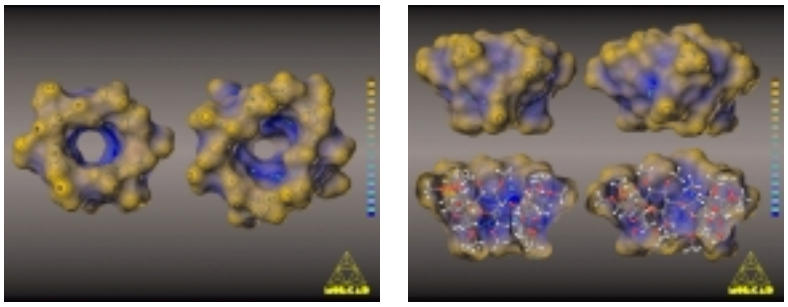

Figure 1.12: MLPs of permethylated α- and β-CDs
Top view (left entry) depicting the highest hydrophobicity at the secondary O2–CH_3, O3–CH_3 methoxyls side of the macrocycle. Side view (right entry, O6–CH_3 groups direct downward) showing the outer surface areas (top ones) which are also mainly hydrophobic (yellow) and the inner regions (bottom ones) which are more hydrophilic (blue).

1.2.2.1 Negative Solubility Coefficient

A unique property of methylated CDs is their "negative" solubility coefficient in water [166, 183], i.e., they are very well soluble in cold water and only slightly soluble in hot water where they usually precipitate or crystallize. This contrasts sharply the normal solubility behavior found in organic compounds. Figure 1.13 shows the solubility as a function of the temperature of methylated CDs compared to their parents. At RT, DIMEB is more soluble in water than the others (*ca.* 60 g/100 mL, Table 1.5). On heating, its homogeneous and clear solution crystallizes immediately. The crystallization temperature depends on the concentration, but for a given condition crystallization takes place within 0.5°C range. On cooling the crystals re-

dissolve; this is shown by the hysteresis cycle of DIMEB in the range of 7–12° [167] (Figure 1.14).

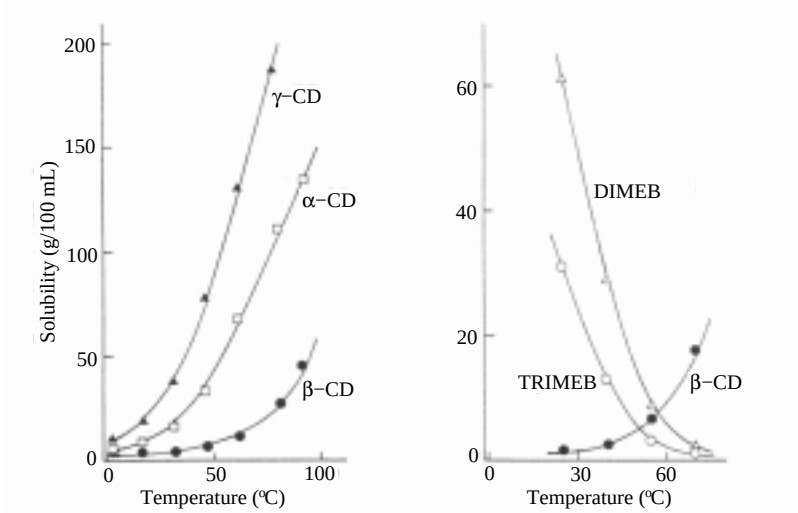

Figure 1.13: Aqueous solubility curves of methylated and native CDs
While the native species have *positive* solubility coefficients (left), the methylated derivatives exhibit *negative* solubility coefficients (right), i.e., the solubility is inversely proportional to the temperature. Taken from [71].

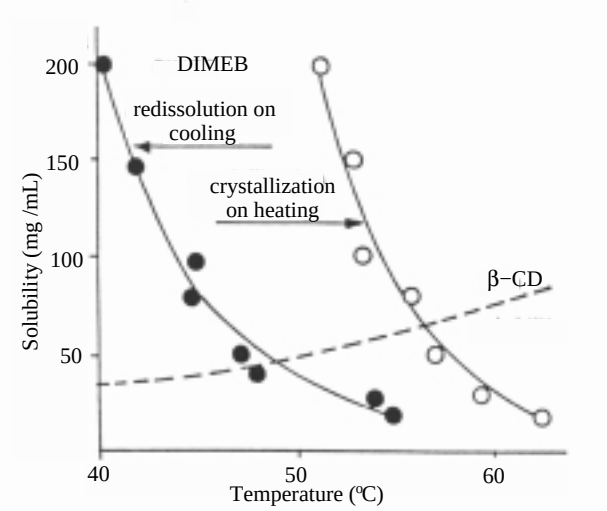

Figure 1.14: Hysteresis loop of DIMEB and solubility of β-CD
Dissolution on cooling and recrystallization on heating of DIMEB showing a hysteresis cycle of 7–12° C [167].

1.2.2.2 Ability to Form Inclusion Complexes

Upon methylation, the inclusion property of CDs with some guest molecules is improved because they better fit to the CD host cavity. This is evidenced by a number of crystal structures of inclusion complexes of methylated and native CDs with the corresponding guest molecules, e.g., carmofur with β-CD [76], with DIMEB [76]; p-iodophenol with β-CD [163], with DIMEB [64, 67], with TRIMEB [80]; 1-propanol with α-CD [138], with DIMEA [65, 69]; 3-iodopropioic acid with α-CD [79], with DIMEA [68]; and recently, acetonitrile with α-CD [10], with DIMEA [6, 8]. Methylated CDs also have higher potential to recognize the chiral guest molecules, e.g., 1-phenylethanol with α-CD [62], with TRIMEA [70]; flurbiprofen with β-CD [181, 182], with TRIMEB [78, 84]; and mandelic acid with TRIMEA [77, 83]. This is due to lack of intramolecular, interglucose O2(n)$\cdots$O3($n-1$) hydrogen bonds in the trimethyl-CD macrocycles which are much less symmetric and can discriminate the chiral compounds better than the symmetric native CDs. The chiral recognition property of methylated CDs are widely used in chromatography [153, 167] to separate racemic mixtures. A conclusion obtained from these crystallographic results is that methylation affects not only the macrocyclic conformation of the host molecule but also the geometry of the host-guest interaction [81, 82]. In addition, the thermodynamic parameters of complex formation in solutions [132] showed that complexes of methylated CDs are more stable than those of native species.

1.2.3 Crystal Structures of Uncomplexed CDs

Because of the negative solubility coefficients, methylated CDs are readily crystallized from hot water where they are less soluble. Therefore, the methylated CDs in both uncomplexed and complexed forms are usually crystallized by dissolving the materials in water at RT or lower temperature, and the solution is then warmed up and left at 40–50°C. To investigate the peculiar aqueous solubility of methylated CDs, they have been crystallized from hot and cold water at 40–80°C and 4–18°C, respectively, and their crystal structures have been determined by X-ray analyses. The following is a summary of structural description of the uncomplexed methylated CDs classified in two groups according to the crystallization temperature.

1.2.3.1 High Temperature Structures

– DIMEA anhydrate crystal structures have been independently determined by Harata [73] and Steiner *et al.* [155]. These crystals are obtained from water at 90 and 80°C, the macrocycles are almost isomorphous with "round" shapes and are stabilized by intramolecular, interglucose O3(n)–H$\cdots$O2($n+1$) hydrogen bonds (Figure 1.15) as generally found in structures of native CDs. Minor difference is the disorder of one O6–CH_3 group. The cavity of DIMEA is not empty but filled by the O6–CH_3 group of a neighboring DIMEA related by the twofold screw axis ("self-inclusion").

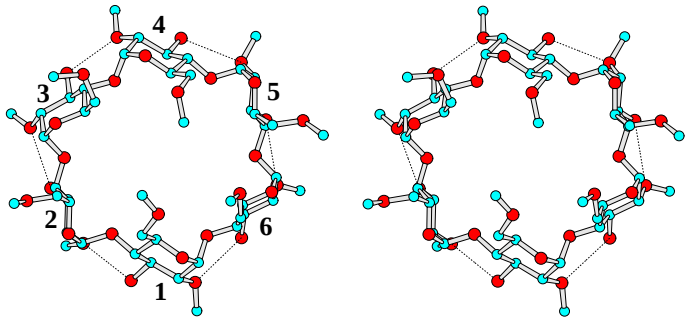

Figure 1.15: Stereo plot of DIMEA anhydrate
DIMEA anhydrate crystallized from water at 80–90°C. The "round" conformation is stabilized by O3(n)–H$\cdots$O2($n+1$) hydrogen bonds (dashed lines) between adjacent glucose units. Two O6–CH_3 groups of glucose units 1 and 4 are rotated "toward" the cavity and close it at this side giving rise to a "bowl" rather than a "torus". Cyan and red spheres represent carbon and oxygen atoms.

– DIMEB anhydrate crystals obtained from water at 60°C [157]. This macrocycle still exhibits the "round" conformation due to existence of circular O3(n)–H$\cdots$O2($n+1$) hydrogen bonds (Figure 1.16). The DIMEB cavity accommodates a doubly disordered O6–CH_3 group of an adjacent DIMEB; three O6–CH_3 groups are rotated "toward" and close the cavity at this side.

– TRIMEA anhydrate crystals grown from water at 40°C [158]. The molecular structure of TRIMEA is distorted from the normal "round" form (Figure 1.17). This is due to steric hindrance of methyl groups at O2-, O3-side consequently, the glucose units 1 and 4 at diametrically opposed positions are more inclined to the molecular cavity to reduce strain in the macrocycle.

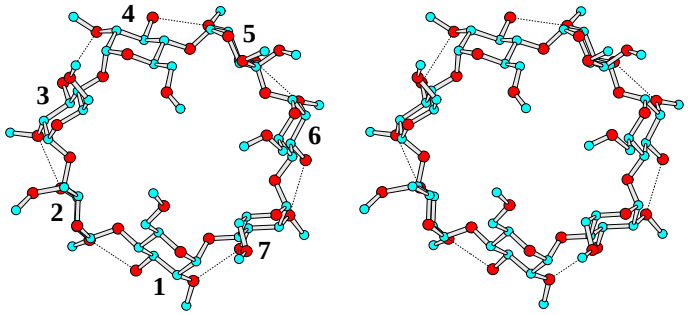

Figure 1.16: Stereo plot of DIMEB anhydrate
DIMEB anhydrate crystallized from water at 60°C. The molecule adopts a "round" conformation and is stabilized by intramolecular, interglucose O3(n)–H$\cdots$O2($n+1$) hydrogen bonds (dashed lines); cyan spheres are carbon atoms and red oxygen atoms.

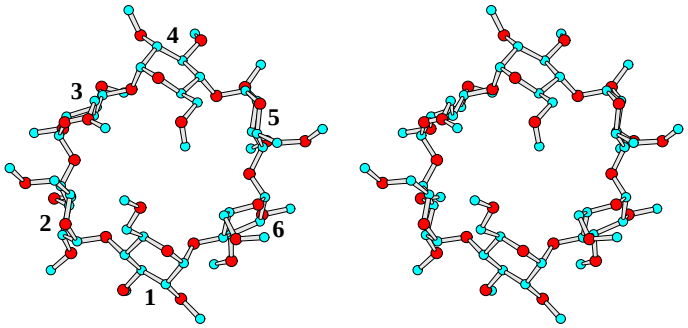

Figure 1.17: Stereo plot of TRIMEA anhydrate
TRIMEA anhydrate crystallized from water at 40°C. The molecule exhibits a distorted "round" conformation with glucose units 1 and 4 tilt strongly to form van der Waals contacts across the cavity. Carbon and oxygen atoms are shown as cyan and red spheres.

– TRIMEB monohydrate crystals grown from water at 50°C [25]. The macrocycle is not "round" anymore but seriously distorted. It adopts an "elliptical" shape with glucose unit 2 in $^1C_4$ chair conformation (Figure 1.18). This is the first time that the inverted chair form could be observed in a structure of CDs or their complexes. One water molecule is located in the intermolecular space between TRIMEB molecules and forms hydrogen bond to O25.

– TRIMEG dihydrate crystals obtained from water at 80°C [160]. To reduce steric strain in the TRIMEG macrocycle, two diametrically opposed glucose units are

flipped by 180° (Figure 1.19). Two water molecules are distributed over four sites outside the TRIMEG cavity and form hydrogen bonds to the O6-CH_3 groups.

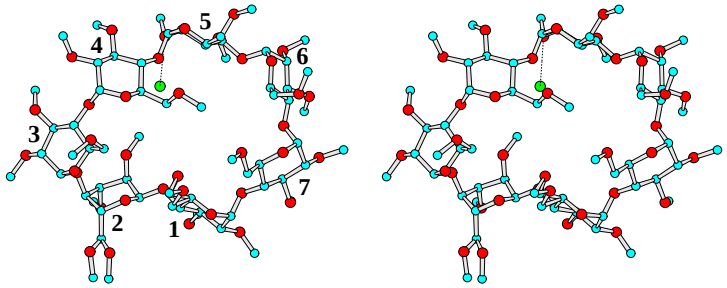

Figure 1.18: Stereo plot of TRIMEB monohydrate
TRIMEB crystallized from water at 50°C as monohydrate. The "elliptical" macrocyclic conformation of TRIMEB is unusual because the glucose unit 2 adopts the inverted $^1C_4$ chair form while the others are in the $^4C_1$ form. One water molecule is located outside the TRIMEB cavity and hydrogen bonds to O25 (see dashed line). Cyan, red and green spheres indicate TRIMEB carbon, oxygen and water oxygen atoms, respectively.

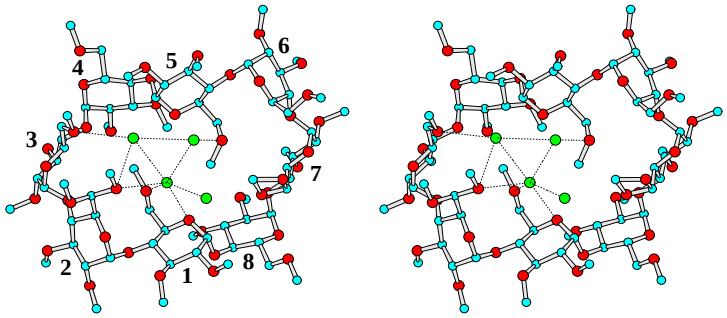

Figure 1.19: Stereo plot of TRIMEG dihydrate
TRIMEG crystallized from water at 80°C as dihydrate. The macrocycle adopts an "elliptical" shape with pseudo-2-fold rotation axis at the center of the TRIMEG cavity. Two diametrically opposed glucose residues 1 and 5 are flipped and their O6–CH_3 groups close the cavity at this side leading to a "bowl" structure. Cyan, red and green spheres indicate TRIMEG carbon, oxygen and water oxygen atoms, respectively. Dashed lines show $O_w \cdots O_{TRIMEG}$ hydrogen bonds.

In summary, the methylated CD crystals grown from hot water show common features: (i) two or three of the O6–CH_3 groups are rotated "inward" and close the

cavity at this side leading to an overall shape of a "bowl" rather than a truncated cone; (ii) the cavity is either empty or filled by the O6–CH_3 group of an adjacent molecule ("self-inclusion"); (iii) none, one, or two water of hydration molecules are located in voids between the CD macrocycles.

1.2.3.2 Low Temperature Structures

– DIMEA monohydrate crystallized from 10% NaCl aqueous solution at RT [73]. The macrocyclic conformation is almost indentical to that of DIMEA anhydrate [73, 155]. One water molecule is disordered over four sites in the central cavity of DIMEA (Figure 1.20). An additional CH_3 group with 48% occupancy is found attached at O32; this is an indication of impurity of the material.

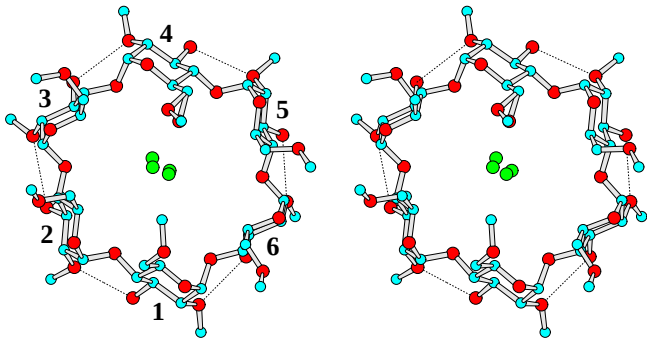

Figure 1.20: Stereo plot of DIMEA monohydrate
DIMEA crystallized from 10% NaCl solution at RT as monohydrate. The "round" molecular conformation is stabilized by O3–H(n)$\cdots$O2(n + 1) hydrogen bonds (dashed lines) and the cavity hosts a water molecule which is disordered over four sites. DIMEA carbon, oxygen and water oxygen atoms are shown as cyan, red and green spheres, respectively.

– DIMEB·$2H_2O$ [11, 12], DIMEB·$15H_2O$ [9]

– (4TRIMEG)·$19.3H_2O$ [13], TRIMEG·$4.5H_2O$ [7]

The four crystal structures of DIMEB and TRIMEG mentioned above, all obtained from water at 18°C. Except for DIMEB·$2H_2O$, these structures show common features of heavy hydration. Their structural details are described in chapter 3 (Results and Discussion).

1.3 Motivation for this Study

Table 1.6 summarizes the crystals of methylated CDs which could be obtained from cold and hot water. In hot water at 40–90°C they crystallized as anhydrate: DIMEA [73, 155], DIMEB [157], TRIMEA [158]; monohydrate: TRIMEB·H_2O [25]; and dihydrate: TRIMEG·2H_2O [160]. The small number of hydration water in these crystals probably correlate to their poor aqueous solubility at high temperature. By contrast, methylated CDs crystallized from cold water at 18°C in heavily hydrated forms, (4TRIMEG)·19.3H_2O [13], TRIMEG·4.5H_2O [7], and DIMEB·15H_2O [9]; this probably explains why methylated CDs are so soluble at these conditions. In one case DIMEB crystallized as dihydrate [11, 12] but this could not be reproduced.

Table 1.6: Known crystal structures of uncomplexed methylated CDs
Methylated CD crystals can be categorized in two groups, one obtained from cold water at 18–25°C, and other one from hot water at 40–90°C. [a] anhy. denotes anhydrate.

Compound		Crystal form/Crystallization temperature (°C)			
		cold water		*hot* water	
Dimethyl	α	DIMEA·H_2O	RT	DIMEA anhy.[a]	80—90
	β	DIMEB·2H_2O	18	DIMEB anhy.[a]	60
		DIMEB·15H_2O	18		
	γ	–	–	–	–
Trimethyl	α	–	–	TRIMEA anhy.[a]	40
	β	–	–	TRIMEB·H_2O	50
	γ	(4TRIMEG)·19.3H_2O	18	TRIMEG·2H_2O	80
		TRIMEG·4.5H_2O	18		

By combination of these crystallographic results, the crystals of methylated CDs obtained from hot and cold water may represent snapshots of their aqueous solutions at both environments. A hypothesis to explain this unusual solubility phenomenon can be stated as follows: at low temperature, the methylated CDs are well soluble because they are strongly hydrated whereas at elevated temperatures where the water and CD molecules become more mobile, hydration water is stripped off and crystallization occurs.

With crystallographic methods alone it is not possible to find the reason for high solubility in cold water and low solubility in hot water of the methylated CDs. Therefore, other techniques which allow to monitor directly solutions of methylated CDs in water need to be integrated. Neutron scattering is an appropriate tool for this purpose because it can provide both structural and dynamical information simultaneously. This is due to the characteristics of neutrons with wavelengths (1–10 Å) and energies (1–100 meV) which are in the order of magnitude of molecular dimensions and motion energies, respectively. Particularly, the incoherent neutron scattering is a unique and powerful technique to elucidate the hydration dynamics of biological molecules [41, 42, 43, 44], due to the high scattering cross-section of the protons. Furthermore, by H_2O/D_2O solvent exchange it is possible to separate the motion of the solute from that of the solvent because the dominant scattering intensity comes from the nonexchangeable H-atoms of the solute. Details of this method are described in the next chapter (Materials and Methods).