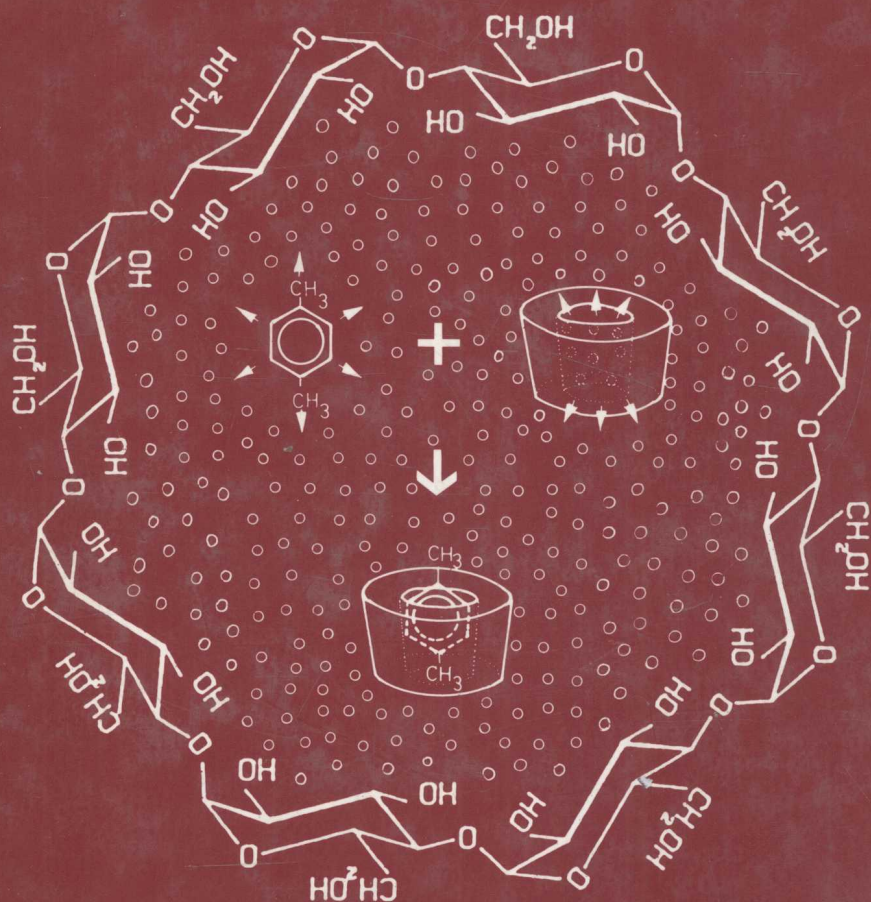

TOPICS IN INCLUSION SCIENCE

József Szejtli

CYCLODEXTRIN TECHNOLOGY



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CYCLODEXTRIN TECHNOLOGY

JÓZSEF SZEJTLI

*Chinoin Pharmaceutical-Chemical Works,
Budapest, Hungary*

捐赠书刊印信

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| 捐赠人 | 何仲贵 |
| 2005 年 11 月 日 | |



KLUWER ACADEMIC PUBLISHERS
DORDRECHT / BOSTON / LONDON



Y2001977

ISBN 90-277-2314-1

Published by Kluwer Academic Publishers,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands

Kluwer Academic Publishers incorporates the publishing
programmes of D. Reidel, Martinus Nijhoff,
Dr W. Junk, and MTP Press.

Sold and distributed in the U.S.A. and Canada
by Kluwer Academic Publishers,
101 Philip Drive, Norwell, MA 02061, U.S.A.

In all other countries, sold and distributed
by Kluwer Academic Publishers Group,
P.O. Box 322, 3300 AH Dordrecht, The Netherlands

2-0392-150 ts

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PREFACE

The long history of cyclodextrins consists of four periods. In the first (from 1891 till mid-thirties of this century) the structure and the industrial potential of cyclodextrins remained unclear.

The first reference to cyclodextrins was made in a publication of Villiers in 1891. From a culture medium of *Bacillus amylobacter*, grown on a medium containing starch, he isolated a small amount of a crystalline substance. He characterised it by its physical data including solubility etc. These were in good agreement with the data published in 1903 by Schardinger for β -cyclodextrin. Villiers named his crystalline product "cellulosine" owing to its alleged similarity to cellulose (e.g. relative stability towards hydrolysis) and observed even then the existence of two different kinds of "cellulosine".

In the course of his studies between 1903 and 1911 on heat resistant micro-organisms responsible for some food poisonings, Schardinger observed that Villiers' cellulosine often formed on a starch-based culture media as a product of putrefying micro-organisms. He succeeded in isolating a bacillus, which he named *Bacillus macerans*, and this is the most frequently used source of the enzyme by which cyclodextrin is now produced.

In the 24 years following Schardinger's last publication, it was Pringsheim who played the leading role in cyclodextrin research. He published extensively although his papers contain several repetitions, contradictions and errors. It is to his credit that he discovered the complexing power of cyclodextrins.

In the second period, from the mid-thirties until the seventies, the structure of cyclodextrin became elucidated by Freudenberg and co-workers. Complex forming properties were studied, numerous derivatives prepared and its enzyme modelling properties discovered. Among many scientists the name of Cramer and French hall-marks this period.

The third period is characterised by a large and explosion-like increasing number of published papers and patents, adequate toxicological studies, and last but not least by the beginning industrial production of CDs.

The fourth period - which is just beginning - is characterised by the second generation of CDs: the industrial production and utilisation of chemically modified CDs. The various BCD derivatives (alkylated, hydroxyalkylated, polymerised etc) open new and unexpected possibilities.

Up to end 1986 about 750 patents (applications) were published, relating to CDs and their applications. The very first patent is dated to 1953 (by Freudenberg, Cramer and Plieninger) which was scarcely followed until the end of the sixties. In the first half of the

seventies the number of patents and patent applications was not more than 10 per annum, and after that rose to 40 p.a. The period 1980-1985 has already seen a rate of 80 p.a.

The increase in the number of published papers is similar. Prior to 1975 less than 50 papers per year were published. In 1979 this number rose to 100, and the number of scientific papers, patents, books, conference lectures in this year (1987) will be well over 400. Keeping in step with this deluge of information is impossible without an up-to-date abstracting service, based on computerised retrieval systems.

Earlier literature on CDs has been summarised by various authors:

1932 by Pringsheim
1941 by Samec
1954 by Cramer
1957 by French
1965 by Thoma and Stewart
1968 by Caesar
1978 by Bender and Komiya
1982 by Szejtli
1987 by Duchéne

The American Cyclodextrin market is predicted to reach 50 million \$ p.a. within two years, with at least twice that if food use is approved by the Food and Drug Administration (C. Eng. News, May 1987). The world market will be at least double that figure. According to the most recent estimation (Bioproc. Technol., Nov. 1987) the potential US market for cyclodextrins is about 32000 tons, 245 million \$ p.a.

The CHINOIN Pharm. Chem. Works launched its CD-research project in 1974, running substantial financial risks, because it was clear that no reward could be expected for ten years. It is to the credit of its then Research Director, the late Prof. Dr. Z. Mészáros, that he supported this project by every means. I should like to express my sincere gratitude to all of my former and present co-workers (their names are listed in the references) whose devoted co-operation made it possible to continue the experimental work through 14 years, and to compile a cyclodextrin literature bank. I am indebted to my friend, James Pagington, for reading and correcting the manuscript of this book.

Budapest, Spring 1988

JÓZSEF SZEJTLI

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CHAPTER 1

CYCLODEXTRINS

1.1 CHEMICAL AND PHYSICAL PROPERTIES

1.1.1 Chemical structures

Enzymic degradation of starch generally results in the production of glucose, maltose, maltotriose, etc., i.e. a long series of linear or branched chain malto-oligomers, known as dextrins.

Dextrins are heterogeneous, amorphous, hygroscopic substances, produced in large quantities for the food, textile, paper and other industries. They are also consumed without prior isolation in such products as e.g. beer and bread. This type of starch degradation is a true hydrolytic process, as the primary product from the splitting of the glycosidic linkage reacts with one molecule of water.

If however, the starch is degraded by the glucosyltransferase enzyme (CGT), the primary product of the chain splitting undergoes an intramolecular reaction without the participation of a water molecule. The α -1,4-linked cyclic products are formed; they are known as cyclodextrins (Figure 1-1).

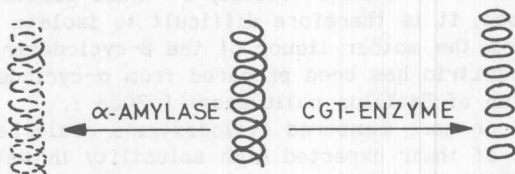


Fig.1-1. Formation of acyclic and cyclic dextrins from starch.

Cyclodextrins are a family of three well known, industrially produced, major cyclic oligosaccharides, and several minor, rare ones. The three major cyclodextrins are crystalline, homogeneous, non-hygroscopic substances, which are of a torus-like macro ring shape, built up from glucopyranose units. The α -cyclodextrin, also known as Schardinger's α -dextrin, cyclomaltohexaose, cyclohexaglukan, cyclohexaamylose, α -CD, ACD and C6A, comprises 6 glucopyranose units. The β -cyclodextrin, also known as Schardinger's β -dextrin, cyclomaltoheptaose, cycloheptaglukan, cycloheptaamylose, β -CD, BCD and C7A, comprises seven glucopyranose units.

The γ -cyclodextrin, also known as Schardinger's γ -dextrin, cyclomalto-octaose, cyclooctaglukan, cyclooctaamylose, γ -CD, GCD and C8A, comprises eight such units (Figure 1-2).

For the historical backgrounds and details of structural features, see the corresponding monographs [16,19,59,82,305,345,346,376].

Sundararajan and Rao [325] demonstrated by conformation-energy map

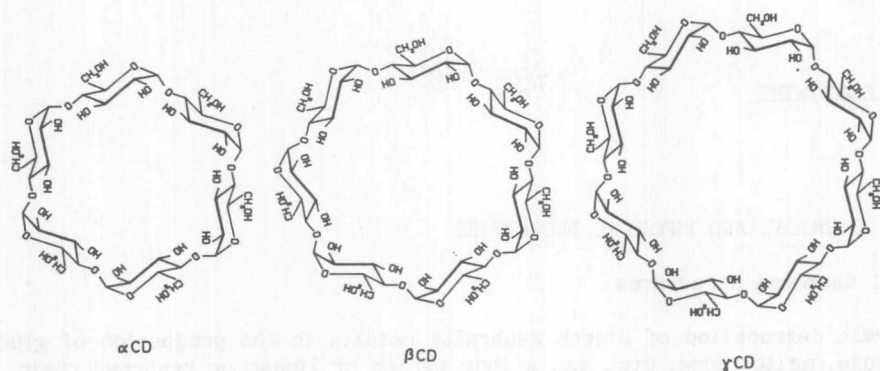


Fig.1-2. Structure of α -, β - and γ -cyclodextrins.

calculations that cyclodextrins with less than six ring members cannot be formed for steric reasons.

Higher homologues have also been reported by Pulley and French [84,300], i.e. δ -, ϵ -, ζ - and η -cyclodextrins. The δ -cyclodextrin is a ring that consists of nine glucose units, however this and the higher ones have not yet been characterised satisfactorily. It is conceivable that they are identical, with the side-chain containing cyclodextrins [178,184] (glucosyl-cyclodextrins) (Figure 1-3). The solubility of these glucosyl-cyclodextrins in water is very good, it is therefore difficult to isolate them in a crystalline form from the mother liquor of the β -cyclodextrin production.

Maltosyl- α -cyclodextrin has been prepared from α -cyclodextrin and maltose by the reverse action of *Bacillus pullulanase* [306a].

Theoretically 10 or more membered cyclodextrins could be prepared, however, on account of their expected high solubility in water, and weak complex forming ability, it seems to be an unrewarding challenge.

The ring of the higher cyclodextrins, if they do exist, will have to be highly flexible. As the apolar cyclodextrin cavity diameter increases, it can

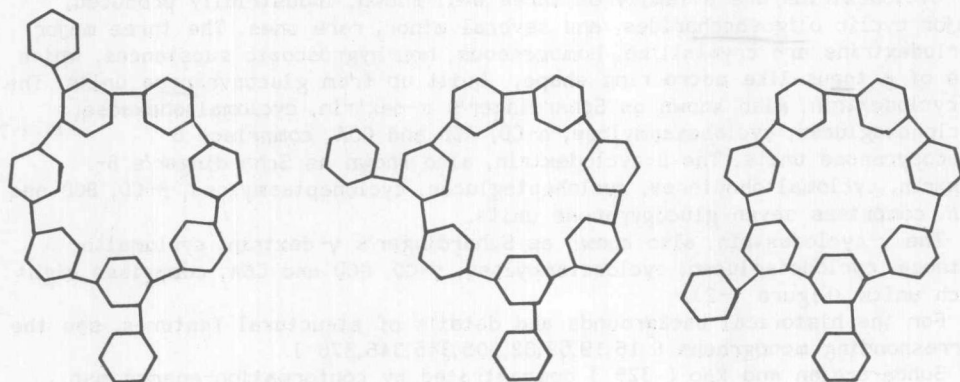


Fig.1-3. Branched di-glucosyl- β -cyclodextrins. [184].

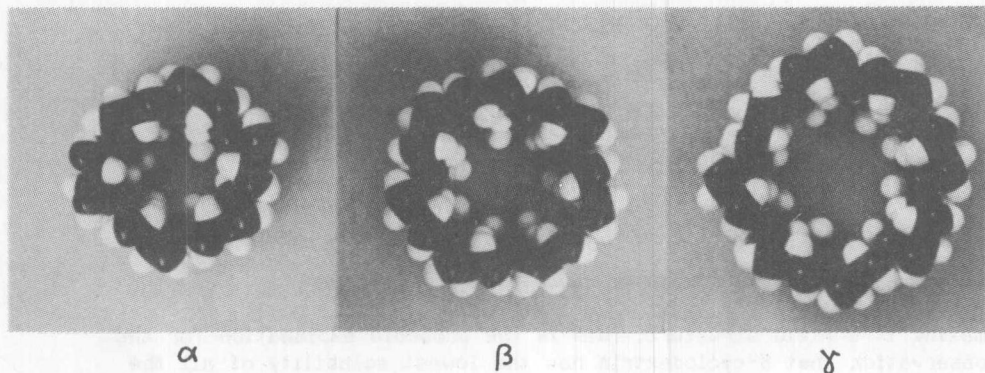


Fig.1-4. Stuart-models of α -, β - and γ -cyclodextrins.

accommodate an increasing number of water molecules, and in aqueous solution these "complexed" water molecules will differ energetically less and less from the bulk of the solvent. As a consequence, complex formation in such a system does not result in a significant gain in energy. Too wide a cyclodextrin cavity will not hold the guest molecules tightly enough to prevent slip-out.

Stuart models of α -, β -, and γ -cyclodextrins, viewed from above, are shown in Figure 1-4. As a consequence of the C1 conformation of the glucopyranose units, all secondary hydroxyl groups are situated on one of the two edges of the ring, and all primary hydroxyls on the other. The cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges, respectively. Both faces of β -cyclodextrin are shown in Figure 1-5. The non-bonding electron pairs of the glycosidic oxygen bridges are directed towards the inside of the cavity, producing a high electron density and lending it some Lewis-base character.

The C2-OH group of one glucopyranoside unit can form a hydrogen bond with the C3-OH group of the adjacent glucopyranose unit [97]. In the cyclodextrin molecule a complete secondary belt is formed by these H-bonds,

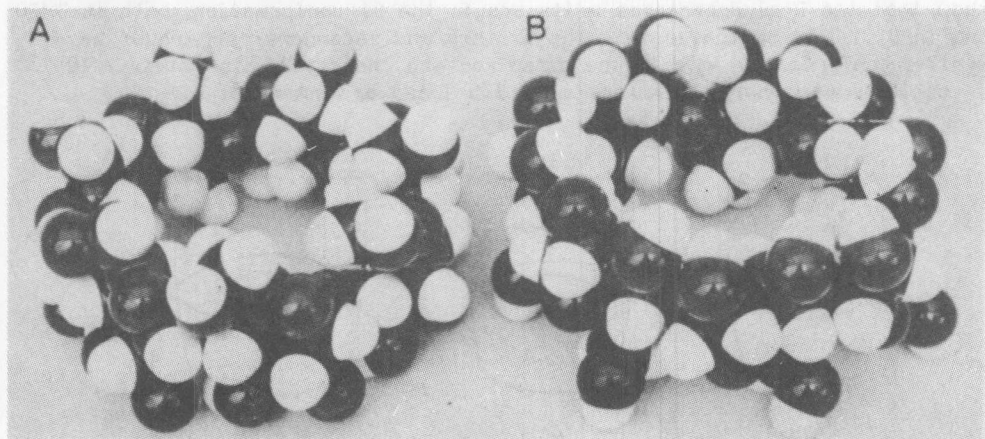


Fig.1-5. The primary (A) and the secondary (B) hydroxyl side of β -cyclodextrin.

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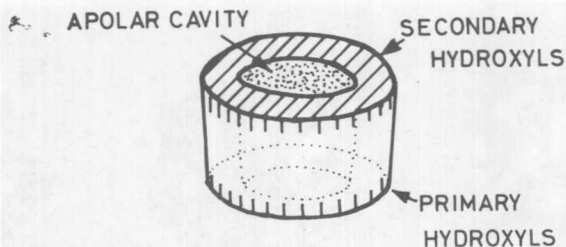


Fig.1-6. Functional structural scheme of cyclodextrins.

making it a rigid structure. This is the probable explanation for the observation that β -cyclodextrin has the lowest solubility of all the cyclodextrins.

The hydrogen-bond belt is incomplete in the α -cyclodextrin molecule, as one glucopyranose unit is in a distorted position [301,304]. As a consequence only four of the six possible H-bonds can be established. The γ -cyclodextrin is a non-coplanar, more flexible structure, and is therefore the more soluble of the three. Equilibrium constants for the hydrogen-deuterium exchange in the secondary hydroxyls (0.65 for β -cyclodextrin, 0.75 for α -cyclodextrin and 0.85 for amylose) indicate the strongest H-bond system to be in β -cyclodextrin [51].

According to conformation-energy maps [325], hydrogen bonding between the C2 and C3 hydroxyls results in a lowering of energy by 20 kcal/mole (83.7 kJ/mole) in α -cyclodextrin, and of 30 kcal/mole (125 kJ/mole) in β -cyclodextrin. Figure 1-6 illustrates the characteristic structural features of cyclodextrins. When involved in inclusion complex formation, cyclodextrin should be regarded as a truncated cone rather than a cylinder.

Free rotation of the primary hydroxyls will reduce the effective diameter of the cavity on the side on which they occur. The approximate dimensions of cyclodextrins are shown schematically in Figure 1-7.

The conformation of cyclodextrins in solution is almost identical to their conformation in the crystalline state. NMR, IR and ORD spectroscopy studies show that the D-glucopyranose units are in the C1 conformation, both in DMSO and in D_2O . This indicates that the primary and secondary -OH groups have similar conformation both in the dissolved and the crystalline state [19].

Conformation energy calculations [325] and a summary of

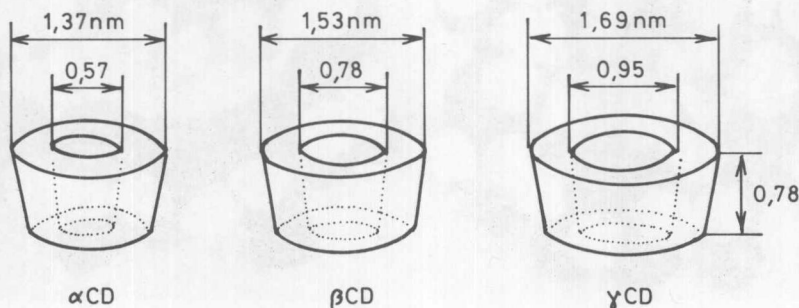
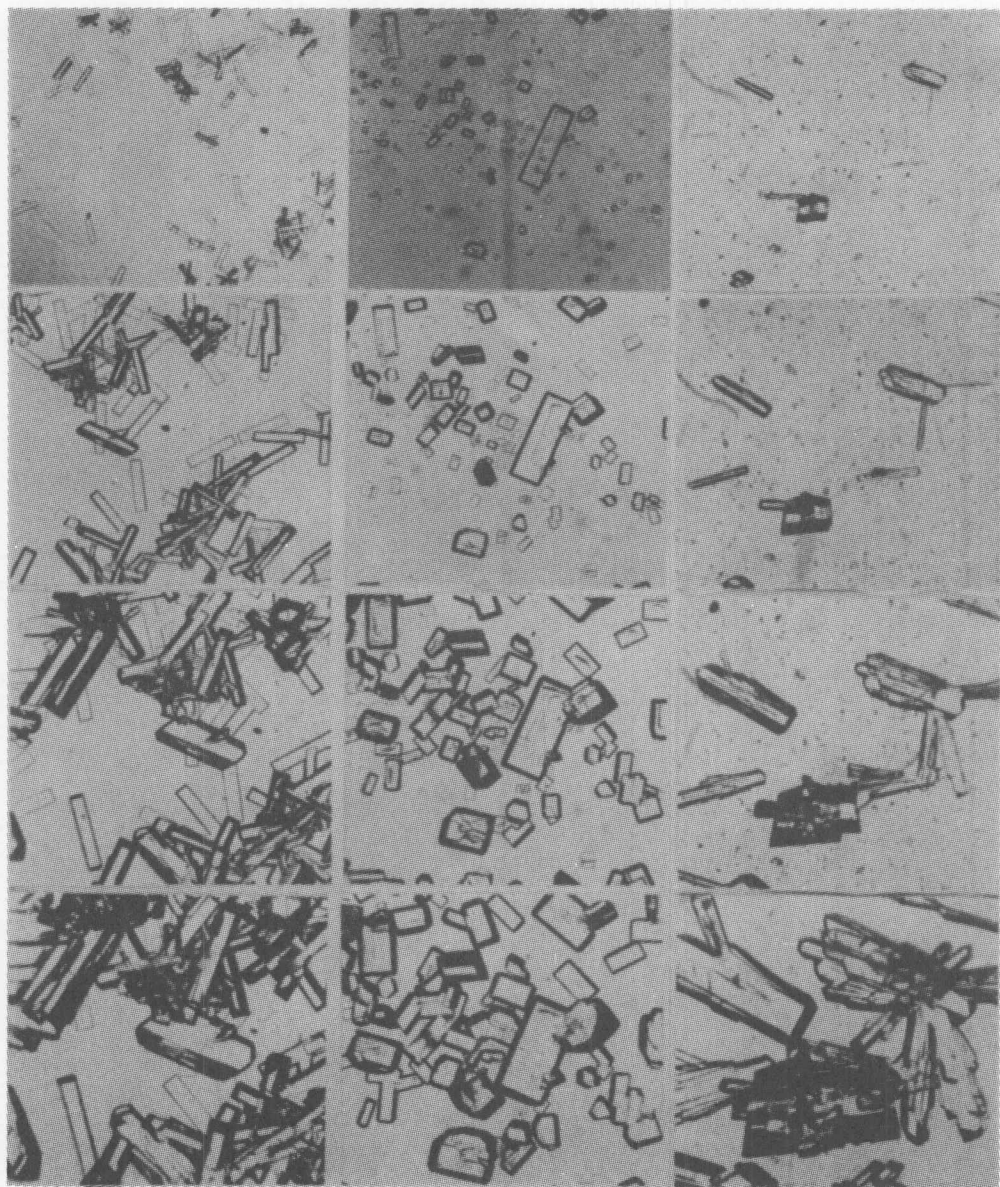


Fig.1-7. Molecular dimensions of cyclodextrins.



α CD 40'
 85'
 120'
 180'

β CD 10'
 20'
 40'
 120'

γ CD 5'
 12'
 25'
 45'

Fig.1-8. Microscopic picture of α -, β - and γ -cyclodextrin (crystallised from water).

crystallographic x-ray studies [305], are published in the corresponding monographs.

1.1.2. Physical properties

1.1.2.1. Crystal structure

Figure 1-8 illustrates the crystals of α - β - and γ -cyclodextrin. The electronmicroscopic picture of thin crystal plates of β -cyclodextrin, at a magnification of 1.5 million, shows a linear structure with a periodicity of 14.5Å. At a magnification of two million, structural units corresponding to cyclodextrin rings can be observed [268].

The packing of cyclodextrin molecules within the crystal lattice occurs in one of two modes: either cage or channel structure [305]. In channel-type structures cyclodextrin molecules are stacked on top of each other; like coins in a roll. The guest molecules are embedded into "endless" channels, formed by the linearly aligned cavities (Figure 1-9). This alignment can be either "head-to-tail" or "head-to-head" type (Figure 1-10). In crystal structures belonging to the cage type, the cavity of one cyclodextrin molecule is blocked off on both sides by adjacent cyclodextrins, thereby producing isolated cavities. In this type of arrangement the cyclodextrin molecules can be packed crosswise, in herringbone fashion (most common in α -, β - and γ -cyclodextrins), or in a brick-wall fashion (Figure 1-9).

The cavities of cyclodextrins crystallised from water are not empty, but filled with water molecules. Some are included into the cyclodextrin cavity, others are integral parts of the crystal structure (crystal water). The cyclodextrin-inclusion complexes are formed by substitution of included water by the appropriate guest molecule.

Depending on the conditions, the same cyclodextrin can result in different crystal forms. Thus α -cyclodextrin has three different crystal forms (α -cyclodextrin·6H₂O, form I; α -cyclodextrin·6H₂O, form II, and α -cyclodextrin·7.57H₂O, form III) [53,207,215]. All are of herringbone structure, as are β -cyclodextrin·12H₂O; β -cyclodextrin·11H₂O; γ -cyclodextrin·13.3H₂O. In α -cyclodextrin·6H₂O only two water molecules are located inside the cavity and four are outside. The two inside molecules are not located on the toroidal axis of the cyclodextrin, but displaced by 0.6Å from this axis. (Figure 1-11) [304].

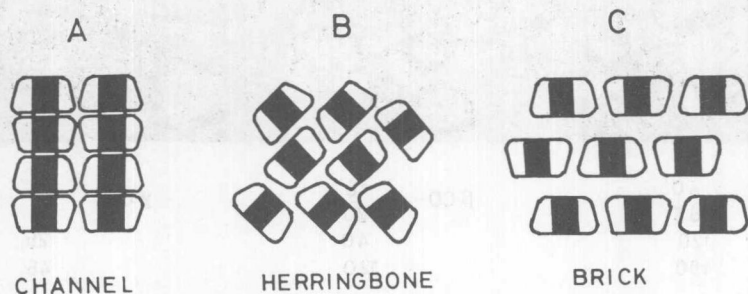


Fig.1-9. Channel (A), herringbone (B) and brick (C) type alignment of cyclodextrin rings in their complex crystal structures [306].

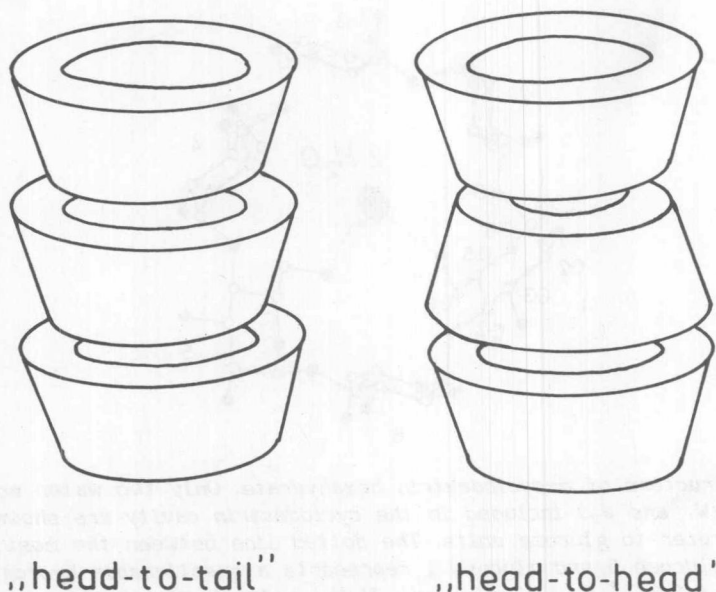


Fig.1-10. "Head-to-tail" and "head-to-head" structures.

The β -cyclodextrin hydrate has two crystal forms: β -cyclodextrin \cdot 12.H₂O and β -cyclodextrin \cdot 11.H₂O [87]. The former one shows a sharp endothermic peak on the DSC or DTA curves at -46°C , whereas the other exhibits a few small peaks between -70 and -60°C . The structural difference between the two forms is found mainly in the distribution of disordered water molecules within the β -cyclodextrin cavity. There are also small differences in the crystal lattice parameters. The undecahydrate is converted into dodecahydrate on standing for a few weeks.

In the β -cyclodextrin \cdot 11H₂O the 11 water molecules are distributed over 16 positions, eight in the cavity (6.12 water molecule), and eight in the interstices (4.88 water molecules). Cavity water forms only two H-bonds to the host- β -cyclodextrin, and six contacts to neighbouring β -cyclodextrins. Their positions are more ordered or "fixed" than the "outside waters" in the interstices. This observation is probably due to the hydrophobic character of the cavity [35].

In the β -cyclodextrin \cdot 12.H₂O the cavity is occupied by 6.5 water molecules, distributed throughout eight positions, and the other 5.5 water molecules are located in the interstices. The included water molecules display intensive thermal motion [212,214].

A β -cyclodextrin molecule with a disordered water structure possesses an unusual "flip-flop" hydrogen bonding system, which displays dynamics, even in the solid state [120].

The γ -cyclodextrin \cdot 13.3H₂O includes 5.3 water molecules, which occupy the 13 sites [107]. Determination of the unit cell and crystal density (corrected for crystal water) in α -cyclodextrin, reveal that there are 24 glucose units in an orthorhombic unit cell of space group P2₁2₁2₁.

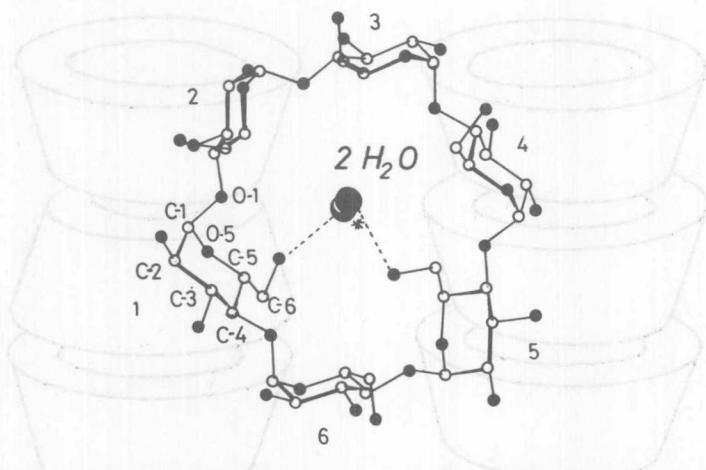


Fig.1-11. Structure of α -cyclodextrin hexahydrate. Only two water molecules (W_1 and W_2) included in the cyclodextrin cavity are shown. Numbers refer to glucose units. The dotted line between the most rotated glucose 5 and glucose 1 represents a questionable hydrogen bond of 3.36 Å O----O distance. Water molecule W_2 is above W_1 , and hydrogen bonded to W_1 and two O(6) hydroxyl groups of α -cyclodextrin. The mark shows the centre of the cyclodextrin molecule. [306].

Dimensions of the unit cell are $a = 14.856$, $b = 33.881$ and $c = 9.717$ Å. The calculated density is 1.493 g cm^{-3} , and the actual density, determined by the flotation method, was 1.491 g cm^{-3} [225, 226].

The unit cell of β -cyclodextrin· $12\text{H}_2\text{O}$ contains 14 glucose units, its space group is $P2_1$, $a = 21.29$, $b = 10.33$, $c = 15.10$ Å [213]. The unit cell of the tetrahedral space group $P4_2$, characteristic of γ -cyclodextrin, is made up of 48 glucose units [82]. Dimensions of the unit cell are $a = 20.25$, $b = 10.95$, $c = 16.89$ Å [222], and 17 water molecules are bonded by one γ -cyclodextrin ring.

These values were determined at about -100°C . Similar studies at 22°C with different crystallisation conditions, resulted in a γ -cyclodextrin· $13.3\text{H}_2\text{O}$ hydrate, where $a = 16.85$, $b = 11.01$ and $c = 20.27$ Å [107].

In this structure the γ -cyclodextrin ring is slightly distorted from the regular octagonal structure. The water molecules in the γ -cyclodextrin cavity are characterised by a high degree of disorder, which is greater than in the β -cyclodextrin· $12\text{H}_2\text{O}$. Many of the disordered water molecules are not fully H-bonded, and seem to be in an "activated state". This may be a significant factor in the inclusion complexation process [107].

As already mentioned, two water molecules are included into the α -cyclodextrin cavity. This causes a distortion in the carbohydrate macroring. One of the glucopyranose units is forced to adopt an out-turned state in relation to the other five units. Its ω and ψ dihedral angles, which characterise the conformation of this unit in the macroring, are 154° and -135° , rather than the average values 166° and -169° respectively. Four C5-