Introduction and General Overview of Cyclodextrin Chemistry

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Introduction

Supramolecular chemistry is that discipline of chemistry which involves all intermolecular interactions where covalent bonds are not established between the interacting species: i.e., molecules, ions, or radicals. The majority of these interactions are of the host−guest type. Among all potential hosts, the cyclodextrins seem to be the most important ones, for the following reasons:

(1) They are seminatural products, produced from a renewable natural material, starch, by a relatively simple enzymic conversion.

(2) They are produced in thousands of tons per year amounts by environmentally friendly technologies.

(3) As a result of point 2, their initially high prices have dropped to levels where they become acceptable for most industrial purposes.

(4) Through their inclusion complex forming ability, important properties of the complexed substances can be modified significantly. This unprecedented "molecular encapsulation" is already widely utilized in many industrial products, technologies, and analytical methods.

(5) Any of their toxic effect is of secondary character and can be eliminated by selecting the appropriate CD type or derivative or mode of application.

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(6) As a result of point 5, CDs can be consumed by humans as ingredients of drugs, foods, or cosmetics.

1. History: The Three Stages in the Development of Cyclodextrin Chemistry

1.1. Discovery Period: From 1891 to the 1930s

The first reference to a substance which later proved to be a cyclodextrin, was published by Villiers,[1] in 1891. Digesting starch with *Bacillus amylobacter* (which probably was not a pure culture, but also contained heat-resistant spores of *Bacillus macerans*), he isolated about 3 g of a crystalline substance from 1000 g starch, and determined its composition to be $(C_6H_{10}O_5)_2 \cdot 3H_2O$. Villiers named this product

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"cellulosine", because it resembled cellulose with regard to its resistance against acidic hydrolysis and because it did not show reducing properties. Even at that time, he observed that two distinct crystalline "cellulosines" were formed, probably α- and β-CDs.

Twelve years later, Schardinger,[2] who studied various isolated strains of bacteria that survived the cooking process and which were thought to be responsible for certain cases of food poisoning, published a report that digesting starch with such a microorganism resulted in the formation of small amounts of two different crystalline products. These substances seemed to be identical with the "cellulosines" of Villiers. Schardinger continued to study these crystallized dextrins, with the expectation that they would shed some light on the synthesis and degradation of starch. He named the isolated microbe *Bacillus macerans*.[3,4] He observed that the crystalline dextrins formed characteristic iodine adducts upon the addition of iodine−iodide solution. He reported that about 25−30% of the starch could be converted to crystalline dextrins (with an additional larger amount of amorphous dextrins). In all of his experiments, the major crystalline product was the so-called α-dextrin. The simplest means to distinguish between the α- and β-dextrins was the iodine reaction. The crystalline α-dextrin/iodine complex in thin layers is blue when damp and gray−green when dry while the crystalline β-dextrin/iodine complex is brownish (red−brown) damp or dry.[5] It can be said that the fundamentals of cyclodextrin chemistry were laid down by Schardinger.

In the 24 years following Schardinger's last CD publication (in 1911), it was Pringsheim[6,7] who played the leading role in cyclodextrin research. He published extensively, with a number of coauthors, but their papers are of limited value. The greatest weakness in these studies lies in the fact that they worked with incompletely separated fractions, and used inadequate methods, e.g., cryoscopic molecular weight determinations. Pringsheim's numerous papers contain many unfounded speculations, and the majority of the published experimental data are unreliable. This group's merit is, however, the discovery that the crystalline dextrins and their acetates have a high tendency to form complexes with various organic compounds.

1.2. Systematic Studies on CDs and Their Inclusion Complexes: From the 1930s to the 1970s

At beginning of the second period, in the 1930s, Freudenberg and co-workers, based partly on their own experiments and partly on observations published by Karrer,[8] Miekeley,[9] and others, came to the conclusion that the crystalline Schardinger dextrins are built from maltose units and contain only α-1,4-glycosidic linkages. Enzymatic hydrolysis,[10] acetolysis with acetyl bromide, and hydrolysis of the permethylated dextrins[11,12] delivered these results. They described the first scheme for the isolation of homogeneous and pure fractions, and in 1936 postulated the cyclic structure of these crystalline dextrins.[13] In 1948−50, the γ-CD had been discovered and its structure elucidated.[14]

With the beginning of the 1950s, two groups, D. French et al.[15] and F. Cramer et al.[16] began to work intensively on the enzymic production of cyclodextrins, on fractionating them to pure components, and on characterizing their true chemical and physical properties. French[15] discovered that there are even larger CDs, while Cramer's group mainly directed their attention toward the inclusion complex forming properties of the cyclic dextrins.

Freudenberg, Cramer, and Plieninger[17] obtained a patent in 1953. In this one and one-half page patent they covered practically all of the most important aspects of the application of CDs in drug formulations. Using several examples, they demonstrated the protection of easily oxidizable substances against atmospheric oxidation, the enhancement of solubility of poorly soluble drugs, the reduction of the loss of highly volatile substances, etc., by cyclodextrin complexation.

The first fundamental review on cyclodextrins was published in 1957 by French.[15] It was followed in 1965 by a monograph by Thoma and Stewart,[18] and in 1968 by Caesar.[19] French's, otherwise excellent, monograph contained the first misinformation on the toxicity of CDs:

> In unpublished attempts to investigate the ability of animals to utilize Schardinger dextrins, B. H. Thomas and D. French fed rats a diet in which a part of the carbohydrate was supplied by highly purified β-dextrin. The animals refused to eat the test diet except in very small quantities and within a week all animals on the ration were dead. Post-mortem examination did not reveal the cause of death.

Nothing has been published about the analysis of the cyclodextrin, which was fed to the rats: organic solvent content? other impurities? percentage of cyclodextrin in the diet? Such fundamental data as the number of rats that were treated, the existence of a control group or information on dosing have never been published. It is well-known that rats have an extremely sensitive sense of smell. They detect toxic substances by smell, and refuse to eat such substances. Since then, thousands of rats have been fed cyclodextrins in rather large doses. Refusal of a CD-containing diet has never been observed. This fact allows one to conclude that there was a rather high level of toxic organic solvent impurity in French's cyclodextrin.

During the following 25 years, until encouraging results of adequate toxicological studies became available, these few lines, cited above, deterred many scientists from developing CD-containing products for human use.

By the end of the 1960s, the methods for the laboratory-scale preparation of cyclodextrins, their structure, physical and chemical properties, as well as their inclusion complex forming properties had been discovered. Summarizing the literature available at that time, the conclusions could be condensed into three points:

(a) Cyclodextrins are very interesting, promising molecules, worth further study, particularly because of their industrial possibilities.

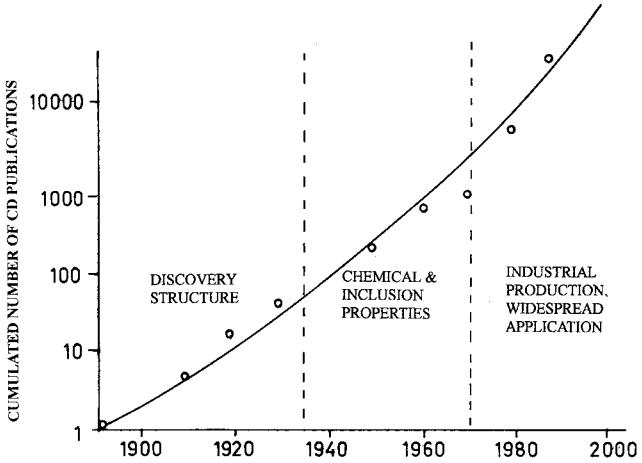

Figure 1. The three stages in development of CD technology. In the first 45 years about 50, in the second period (~35 years) about 2000, and in the third period (during the last 27 years) about 13 000 CD-related publications (papers, patents, conference abstracts) have been published.

(b) Cyclodextrins are very expensive substances, available only in small amounts as fine chemicals.

(c) Cyclodextrins are apparently highly toxic; therefore, their utilizations for human consumption seems to be questionable.

1.3. Industrial Production and Utilization of CDs: From the 1970s Onward

Figure 1 illustrates the logarithmic increase in the number of CD-related publications (including patents, published patent applications, and conference abstracts) during the last century. After adequate toxicological studies proved that any toxicity attributed to CDs originated from complexed impurities, an inadequate form of administration, or extreme dosing, i.e., there is no inherent toxicity of CDs to inhibit their widespread utilization, the number of CD-related publications displayed an explosion-like increase.

The first International Symposium on Cyclodextrins was organized in 1981. From 1984 onward, an International CD symposium has been held every second year with 120−150 lectures summarizing recent results.[20−27] It is estimated that the total number of CD-related publications will be over 15 000 by the end of 1997.[28] While in 1970 the price of 1 kg of β-CD was around $2000 US, and it was available only as a rare fine chemical, 25 years later, worldwide more than half a dozen companies are producing cyclodextrins. Their total output is in excess of 1000 tons/year, and the price of the key product, β-CD is only several dollars per kilogram, depending on quality and delivered quantity. Also α- and γ-CDs, as well as several derivatives, (hydroxypropyl- β-CD and γ-CD, randomly methylated α- and β-CD, maltosyl-β-CD, acetylated CDs, etc.) are produced industrially. A large number of other derivatives are available as fine chemicals, and used in various chromatographic methods, or are studied as potential drug carriers, stabilizers, catalysts, etc.

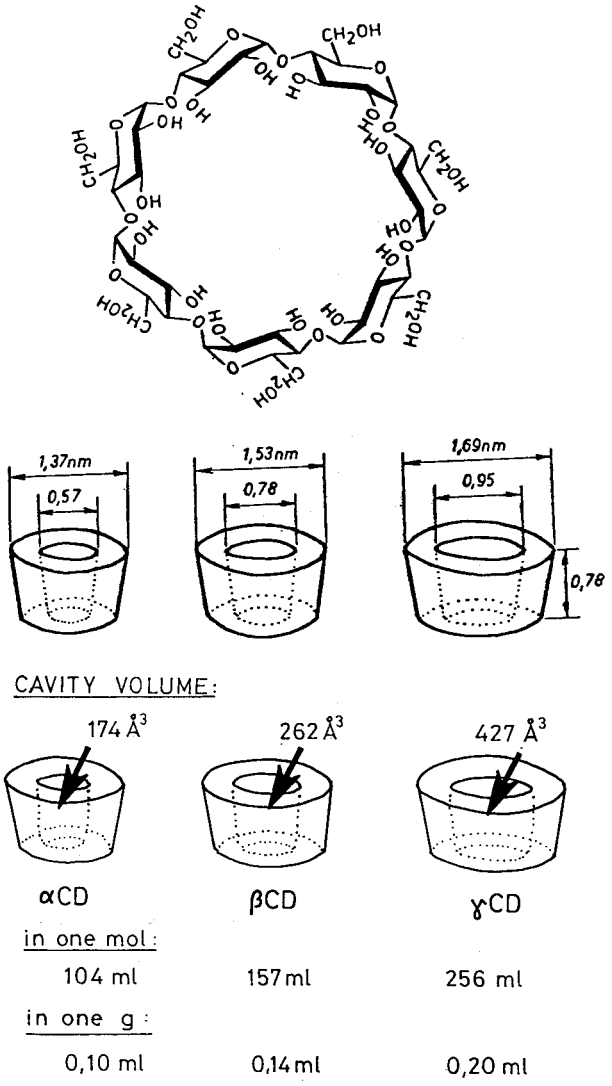

Figure 2. Structure of β-CD and approximate geometric dimensions of α-, β-, and γ-CD molecules.

2. Fundamentals of CD Chemistry

2.1. Structural Features

Cyclodextrins comprise a family of three well-known industrially produced major, and several rare, minor cyclic oligosaccharides. The three major cyclodextrins are crystalline, homogeneous, nonhygroscopic substances, which are torus-like macro-rings built up from glucopyranose units. The α-cyclodextrin (Schardinger's α-dextrin, cyclomaltohexaose, cyclohexaglucan, cyclohexaamylose, α-CD, ACD, C6A) comprises six glucopyranose units, β-CD (Schardinger's β-dextrin, cyclomaltoheptaose, cycloheptaglucan, cycloheptaamylose, β-CD, BCD, C7A) comprises seven such units, and γ-CD (Schardinger's γ-dextrin, cyclomaltooctaose, cyclooctaglucan, cyclooctaamylose, γ-CD, GCD, C8A) comprises eight such units (Figure 2). The most important characteristics of the CDs are summarized in Table 1.

The nomenclature of CDs is not exact. Maltose is a disaccharide, i.e., a cyclomaltopentaose could be interpreted as a 10 glucopyranose containing cyclic oligosaccharide. Otherwise, this is the five-membered pre-α-CD. How would a four-membered CD be named?

Table 1. Characteristics of α-, *β*-, and *γ*-CDs[35]

	α	*β*	*γ*
no. of glucose units	6	7	8
mol wt	972	1135	1297
solubility in water, g 100 mL^{-1} at room temp	14.5	1.85	23.2
$[\alpha]_D$ 25 °C	150 ± 0.5	162.5 ± 0.5	177.4 ± .5
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 outher periphery, Å	14.6 ± 0.4	15.4 ± 0.4	17.5 ± 0.4
approx volume of cavity, $Å^3$	174	262	427
approx cavity volume in 1 mol CD (ml)	104	157	256
in 1 g CD (ml)	0.10	0.14	0.20
crystal forms (from water)	hexagonal plates	monoclinic parallelograms	quadratic prisms
crystal water, wt %	10.2	13.2−14.5	8.13−17.7
diffusion constant at 40 °C	3.443	3.224	3.000
hydrolysis by *A. oryzae* α-amylase	negligible	slow	rapid
V_{max} value, min^{-1}	5.8	166	2300
relative permittivity (on incorporating the toluidinyl group of 6-*p*-toluidynilnaphthalene 2-sulfonate) at pH = 5.3, 25 °C	47.5	52.0	70.0
(on incorporating the naphthalene group)	*a*	29.5	39.5
p*K* (by potentiometry) at 25 °C	12.332	12.202	12.081
partial molar volumes in solution mL mol^{-1}	611.4	703.8	801.2
adiabatic compressibility in aqueous solutions mL (mol^{-1} bar^{-1}) × 10^4	7.2	0.4	−5.0

[a] Naphthalene group is too bulky for the α-CD cavity.

Fortunately, the practically important, industrially produced CDs are the α-, *β*-, and *γ*-CDs. Their names are unambiguous, and need not be changed. A complete and unanimous nomenclature has been suggested by Lichtenthaler and Immel,[27,29] e.g. the pre-α-CD is named as cyclo-α(1→4)-glucopentaoside. This nomenclature is recommended for the so-called minor CDs, as well as for any cyclic oligosaccharides.

As a consequence of the $^4C_1$ conformation of the glucopyranose units, all secondary hydroxyl groups are situated on one of the two edges of the ring, whereas all the primary ones are placed on the other edge. The ring, in reality, is a cylinder, or better said a conical cylinder, which is frequently characterized as a doughnut or wreath-shaped truncated cone. The cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges, respectively. The nonbonding electron pairs of the glycosidic oxygen bridges are directed toward the inside of the cavity producing a high electron density there and lending to it some Lewis base characteristics.

The C-2-OH group of one glucopyranoside unit can form a hydrogen bond with the C-3-OH group of the adjacent glucopyranose unit. In the CD molecule, a complete secondary belt is formed by these H bonds, therefore the *β*-CD is a rather rigid structure. This intramolecular hydrogen bond formation is probably the explanation for the observation that *β*-CD has the lowest water solubility of all CDs.

The hydrogen-bond belt is incomplete in the α-CD molecule, because one glucopyranose unit is in a distorted position. Consequently, instead of the six possible H-bonds, only four can be established fully. The *γ*-CD is a noncoplanar, more flexible structure; therefore, it is the more soluble of the three CDs.

Figure 3 shows a sketch of the characteristic structural features of CDs. On the side where the secondary hydroxyl groups are situated, the diameter of the cavity is larger than on the side with the primary hydroxyls, since free rotation of the latter

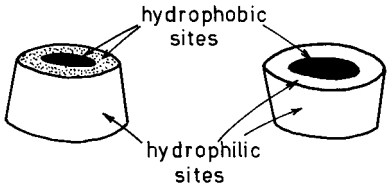

Figure 3. Schematic representation of the hydrophobic and hydrophilic regions of an α-CD cylinder.

reduces the effective diameter of the cavity. The approximate dimensions of CDs are shown schematically in Figure 2.

For a long time, only the three parent (or major) CDs (α-, *β*-, and *γ*-CD) were known and well characterized. French,[15] in the early 1950s, observed the existence of some larger CDs (*δ*, *ϵ*, etc.), however, at that time, it was not yet clear whether they were really 9, 10, etc., membered rings, or glucosyl-, maltosyl-, or diglucosyl-branched CDs.

During the past decade, a series of the larger CDs has been isolated and studied.[30] For example, the nine-membered *δ*-CD was isolated from the commercially available CD conversion mixture by chromatography. The *δ*-CD had greater aqueous solubility than the *β*-CD, but less than that of α- and *γ*-CD. It was the least stable among the CDs known at that time; their hydrolysis rate increases in the order of α-CD < *β*-CD < *γ*-CD < *δ*-CD. The *δ*-CD did not show any significant solubilization effect on slightly soluble drugs in water, except in the cases of some large guest molecules such as spironolactone and digitoxin, e.g. the solubility of spironolactone increased about 30-fold in the presence of *δ*-CD (30).

Table 2 illustrates the complex-forming ability of the larger CDs. These results are in accord with the results of computer graphic studies. The larger CDs are not regular cylinder shaped structures. They are collapsed, and their real cavity is even smaller than in the *γ*-CD (Figure 4). The driving force of the complex formation, the substitution of the high

Table 2. Inclusion Complexing Capacity of α- to *θ*-CDs, Studied by Capillary Electrophoresis[30]

compound	inclusion complex formation constant (M^{-1})							
	α-CD	*β*-CD	*γ*-CD	*δ*-CD	*ϵ*-CD	*ξ*-CD	*η*-CD	*θ*-CD
benzoic acid	16	23	3	3	3	5	4	5
2-methylbenzoic acid	13	13	7	6	6	5	6	7
3-methylbenzoic acid	26	40	6	3	5	6	7	8
4-methylbenzoic acid	36	66	8	2	4	6	6	7
2,4-dimethylbenzoic acid	45	42	8	3	4	5	7	6
3,5-dimethoxybenzoic acid	47	63	10	8	9	10	9	12
salicylic acid	11	65	13	9	8	8	9	10
3-phenylpropionic acid	35	79	7	2	3	5	4	6
4-*tert*-butylbenzoic acid	51	457	59	60	4	9	19	31
ibuprofen	55	2600	59	1013	–	10	44	225
1-adamantanecarboxylic acid	114	501	42	8	–	4	4	8

Figure 4. "Collapsed cylinder" structure of the *δ*-CD.

enthalpy water molecules in the CD cavity, is weaker in the case of larger CDs; therefore, their utilization as inclusion complexing agents will probably remain rather restricted.

The voluminous knowledge accumulated on structural features, physical, chemical, and biological properties and effects of CDs will be treated partly in the specific sections of this review, or can be found in various monographs (e.g. refs 31−36).

2.2. Production of CDs

The cyclodextrin glucosyl transferase enzyme (CGT-ase) is produced by a large number of microorganisms, like *Bacillus macerans*, *Klebsiella oxytoca*, *Bacillus circulans*, and *Alkalophylic bacillus* No. 38-2, etc. Genetic engineering has provided more active enzymes, and probably, in the future, mostly these enzymes will be used for industrial CD production.

The first step in CD production is the liquefaction of the starch at elevated temperature. To reduce the viscosity of the rather concentrated (around 30% dry weight) starch solution, it has to be hydrolyzed to an optimum degree. The prehydrolyzed starch must not contain glucose, or low molecular oligosaccharides, because they strongly reduce the yield of the formed CDs. After cooling to the optimum temperature, CGT-ase enzyme is added to the starch solution. In the so-called nonsolvent technology, the α-, *β*-, and *γ*-CDs formed have to be separated from the complicated partially hydrolyzed mixture. In the case of the solvent technology, an appropriate complex-forming agent is added to the conversion mixture. If toluene is added to this system, the formed toluene/*β*-CD complex is separated immediately, and the conversion is shifted toward the formation of *β*-CD. If 1-decanol is added to the conversion mixture, mainly α-CD will be produced, while in case of cyclohexadecenol the main product is *γ*-CD. Various other complex-forming agents can be used. The selection depends on price, toxicity, and explosivity, but mainly on the efficiency of the removal of the solvents from the crystalline end product. The insoluble complexes are separated from the conversion mixture by filtration. The removal of the solvents from the filtered and washed complex is generally made after suspending it in water by distillation or extraction. The aqueous solution obtained after removal of the complexing solvent is treated with activated carbon and filtered. The cyclodextrins are then separated from this solution by crystallization and filtration. The purity of the industrially produced cyclodextrins is generally better than 99%. Table 3 illustrates their quality.

2.3. CD Inclusion Complexes

In an aqueous solution, the slightly apolar cyclodextrin cavity is occupied by water molecules which are energetically unfavored (polar−apolar interaction), and therefore can be readily substituted by appropriate "guest molecules" which are less polar than water. (Figure 5.) The dissolved cyclodextrin is the "host" molecule, and the "driving force" of the complex formation is the substitution of the high-enthalpy water molecules by an appropriate "guest" molecule. One, two, or three cyclodextrin molecules contain one or more entrapped "guest" molecules. Most frequently the host:guest ratio is 1:1. This is the essence of "molecular encapsulation".

This is the simplest and most frequent case. However 2:1, 1:2, 2:2, or even more complicated associations, and higher order equilibria exist, almost always simultaneously.

The formed inclusion complexes can be isolated as stable crystalline substances. Upon dissolving these complexes, an equilibrium is established between dissociated and associated species, and this is expressed by the complex stability constant K_a. The association of the CD and guest (D) molecules, and the dissociation of the formed CD/guest complex is governed by a thermodynamic equilibrium.

$$\mathrm{CD} + \mathrm{D} \rightleftharpoons \mathrm{CD{\cdot}D} \tag{1}$$

$$K_{1:1} = \frac{[\mathrm{CD{\cdot}D}]}{[\mathrm{CD}][\mathrm{D}]} \tag{2}$$

The most important primary consequences of the interaction between a poorly soluble guest and a CD in aqueous solution are as follows:

• The concentration of the guest in the dissolved phase increases significantly, while the concentration of the dissolved CD-decreases. This latter point is not always true, however, because ionized guests, or

Table 3. Quality Specification of Commercially Available α-, *β*-, and *γ*-CDs of Pharmaceutical Quality (from Wacker-Chemie, Munich)

	α-CD	*β*-CD	*γ*-CD
appearance	white crystalline powder	white crystalline powder	white crystalline powder
content	98% min.	98% min.	98% min.
other cyclodextrins	0.5% max.	0.5% max.	0.5% max.
residue on ignition	0.1 max.	0.1 max.	0.1 max.
specific rotation in aqueous solution $[\alpha]_D$	(+) 148° ± 3°	(+) 161° ± 3°	(+) 173° ± 3°
optical density of a 10% solution at 420 nm	0.1 max.	1% aqueous solution is clear and colorless	0.2 max.
recuding compounds (determined as dextrose)	0.5% max.	0.5% max.	0.5% max.
heavy metals	5 ppm max.	5 ppm max.	5 ppm max.
volatile organics	50 ppm max.	50 ppm max.	50 ppm max.
water (Karl Fischer)	11% max.	14% max.	11% max.
microorganisms	1000/g max.	1000/g max.	1000/g max.
salmonella/*E.Coli*	negative	negative	negative

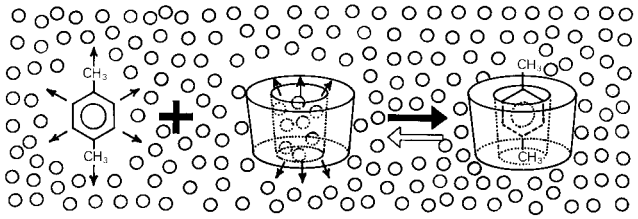

Figure 5. Schematic representation of CD inclusion complex formation. *p*-Xylene is the guest molecule; the small circles represent the water molecules.

hydrogen-bond establishing (e.g. phenolic) compounds may enhance the solubility of the CD.

• The spectral properties of the guest are modified. For example, the chemical shifts of the anisotropically shielded atoms are modified in the NMR spectra. Also when achiral guests are inserted into the chiral CD cavity, they become optically active, and show strong induced Cotton effects on the circular dichroism spectra. Sometimes the maximum of the UV spectra are shifted by several nm and fluorescence is very strongly improved, because the fluorescing molecule is transferred from the aqueous milieu into an apolar surrounding.

• The reactivity of the included molecule is modified. In most cases the reactivity decreases, i.e., the guest is stabilized, but in many cases the CD behaves as an artificial enzyme, accelerating various reactions and modifying the reaction pathway.

• The diffusion and volatility (in case of volatile substances) of the included guest decrease strongly.

• The formerly hydrophobic guest, upon complexation, becomes hydrophilic; therefore its chromatographic mobility is also modified.

And in the solid state:

• The complexed substance is molecularly dispersed in a carbohydrate matrix, forming a microcrystalline or amorphous powder, even with gaseous guest molecules.

• The complexed substance is effectively protected against any type of reaction, except that with the CD hydroxyls, or reactions catalyzed by them.

• Sublimation and volatility are reduced to a very low level.

• The complex is hydrophilic, easily wettable, and rapidly soluble.

When, in an aqueous system, the formation of the CD inclusion complex can be detected, e.g. by NMR or circular dichroism, or through a catalytic effect; this does not mean that a well-defined crystalline inclusion complex can be isolated. The two main components of the driving force of the inclusion process are the repulsive forces between the included water molecules and the apolar CD cavity on one hand, and between the bulk water and the apolar guest, on the other hand. This second factor does not exist in the crystalline (dry) state. Therefore it is not uncommon that the complex formation is convincingly proved in solution, but nevertheless the isolated product is nothing other than a very fine dispersion of the CD and the guest.

3. The CD Literature

The CD literature delivers an excellent example of the problem, which at beginning of the third millennium will represent the major difficulty for scientists: to find, read, and correctly interpret all the literature, which is pertinent to his/her research project. To locate the sources is easy. The computerized databases or reference lists of earlier reviews and monographs contain, with an acceptable probability, all of the relevant literature. Photocopies can be acquired from the most remote libraries of the world, but reading cannot be spared. One needs time, a lot of time, frequently requiring knowledge of various languages, and, without having one's own experimental experience, it is difficult to separate the really original, important papers from the deluge of "me-too" papers. With due prudence, at least 50% of the publications of the two past decades are redundant and unnecessary. They contain nothing new, or even worse, very far reaching conclusions are drawn from observed marginal, insignificant small effects, promising unattainable industrial potentials.

Sometimes, and with increasing frequency, very interesting works are published, having only one defect; the reported phenomena or products have been published 15−25 years earlier, but the authors of the new publication did not find, read, or cite the earlier one.

By end the of 1997, the number of CD-related publications will be more than 15 000, representing more than 100 000 printed pages. The productivity of many scientists, mainly at universities and academic laboratories, is evaluated by the number of

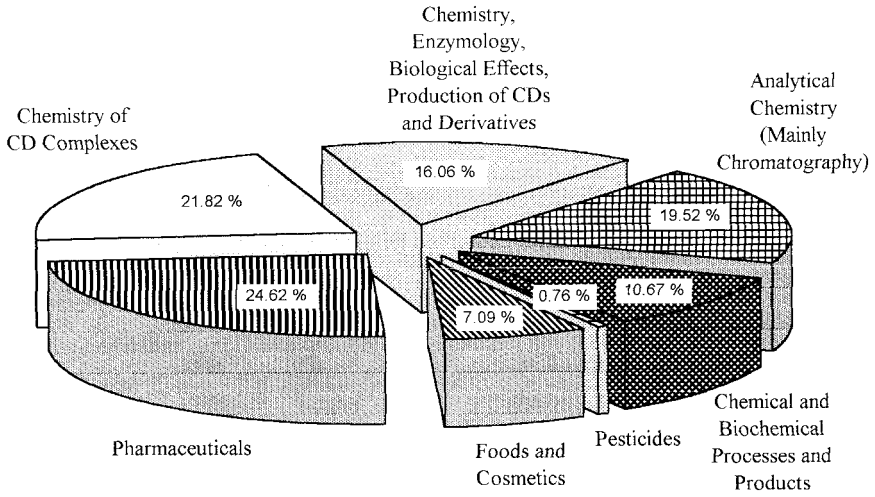

Figure 6. Distribution of the 1706 CD relevant abstracts published in 1996 by *Cyclodextrin News*.

their publications. Of course, not (only) the number of publications is taken into account, other criteria are also important, e.g. the impact factor of the journal, citation frequency, etc., but the first measure of an academic researcher was, and remains, the number of his/her publications. This constraint to publish is the most important driving force in the explosion-like increase of the literature, which makes it practically impossible to read all of the relevant publications, even restricted to a relatively narrow section of the CD literature. The only way to exploit the enormous potential hidden in the vast amount of literature is to summarize it in specific, well-limited critical reviews. More than 450 reviews have been published on cyclodextrins. Less than 10% of them can be considered to be "critical evaluations". The majority of them are nothing but an uncritical compilation of that literature—frequently only as abstract—that the author was able to find and read, mixing up significant, industrially important observations and products, with unfounded speculations and nonfeasible ideas. While 25 years ago about 4−5 CD papers were published monthly, in 1997 just that many are published—daily.

Figure 6 illustrates the classification of the CD papers, according to their subject, abstracted by *CD-News* in 1996.[28]

About 16% of all CD-relevant publications are dedicated to the fundamentals of cyclodextrin chemistry and technology, i.e., the physical and chemical properties of cyclodextrins, their enzymology, toxicology, production, and derivatives. This section also includes the numerous review articles on CDs. With little effort, any microbiologist is able nowadays to discover a new CTG-ase enzyme producing microorganism, but considering that the industrial conversion of starch to cyclodextrins is made by enzymes produced by highly productive mutations of selected microorganisms, or by genetically modified ones, the probability of the practical utilization of these works (at least 2−3 new such publications monthly) is rather low. Papers, reporting on the preparation of cyclodextrins from various starch sources such as potato, sago, various food industry byproducts, and tropical starch sources, are not relevant for practical application, because cyclodextrins have to be produced on a thousand ton/year scale. Otherwise their price is not competitive with that of the large producers.

Nearly 22% of the publications are dedicated to studies of the CD inclusion phenomena. These works are generally not directly practice-oriented, dealing with energetics and kinetics of inclusion, X-ray, FT-IR, liquid and solid-phase NMR, EPR, circular dichroism, Raman spectroscopy, enhancement of luminescence and phosphorescence, thermal analysis, interaction of CDS with specific guest types, enzyme modeling with CDs and CD derivatives, preparation, analysis of cyclodextrin complexes, etc. These methods, as well as the correlation between the complexation and various structural and external parameters form the basis for all practical applications of CDs.

The largest group of CD papers, nearly 25%, is dedicated to the pharmaceutical application of CDs. The majority of drug molecules are poorly soluble in water, consequently their biological absorption is slow and frequently far from being complete. Moreover many drug molecules are rather sensitive to oxidation, thermodecomposition, light, ions, other ingredients of the pharmaceutical formulation, etc. Most drug molecules are ideal complex-forming partners for cyclodextrins, because their polarity, molecular mass, and structure enable them to get included into the CD cavity. This is a very productive field, and considering the lengthy development and strict requirements for approval of a new chemical entity (a cyclodextrin complex of a well-known drug molecule is always considered to be a new chemical entity) it must be considered as a significant achievement that more than a dozen drugs are already approved and marketed in cyclodextrin-complexed form. In the coming years this field will display the most intensive development. Nevertheless, the large number (more than 4000) of drug/CD related papers and patents is a little misleading, because many authors publish the same results in different journals under different titles, but with virtually identical content. Rediscoveries are published frequently, simply because the authors did not read the earlier literature; in essence they have discovered something that was published earlier. Not every cyclodextrin or cyclodextrin derivative can be administered to

humans, partly because some of those cyclodextrins have such a high affinity toward the cell-membrane lipid components of the organism that depending on their concentration, may result in hemolysis, or else their synthesis is too expensive. For example it has not made too much sense to develop a drug/trimethyl-β-CD complex for parenteral application. Because of the many repetitions, and the nonfeasible ideas, only about 30% of the published papers disclose really new and significant results.

Actually only 7% of the CD-related papers are dedicated directly to the food, cosmetic, and toiletry applications of CDs, but at the same time about 70% of all cyclodextrins produced are used in this field.[28,35−37] The approval process for CD-containing products in this field is much simpler and faster that in the case of the drug/CD formulations. The amount of CD used in a cosmetic or toiletry product might be larger, by orders of magnitude, than the amount used in a drug. For example, a well-known drug/CD complex is the prostaglandin E1/α-CD complex, but an ampule contains only 20 μg of prostaglandin E1 and 646 μg of α-CD. Consequently, less that 1 kg of α-CD is needed for the production of one million prostaglandin ampules. For the production of other successful drug/CD complexes such as Brexin (Piroxicam/β-CD complex), which is marketed in many countries of the world, not more than 40−50 ton/year of β-CD is needed. However, a single toiletry product, like a fragrance tissue, which needs no health authority approval, because it is not consumed by humans and is used only in laundries, needs hundred of tons of β-CD every year. Many tons of β-CD, are used for example, for the production of the low cholesterol butter, where the β-CD is used to specifically remove the cholesterol from the milk fat.

Actually, the application of CDs in pesticide formulations is very modest. The relevant publications represent less than 1% of the CD literature. In the pesticide formulations, practically the same effects can be attained by CDs as in the drug formulations. The pesticide industry is, however, very raw material price sensitive, and until now the price of even the cheapest technical-quality β-CD was simply too high for pesticide formulations. This situation, however, will change very soon, because within a couple of years the price of technical-quality β-CD, which will be perfectly acceptable for the pesticide formulation industry, will drop to less than $4 per kilogram. If the pesticide-formulating industry becomes aware of this possibility, a new several thousand ton section of the cyclodextrin market will be opened.

Presently, about 11% of the CD literature is dedicated to the application of CDs in the chemical and biotechnological industries. This section is also expected to display a rapid increase. Particularly in the biotechnology of poorly water soluble substances such as lipids, steroids, etc., the possibility of very rewarding CD applications is already known.[35,36]

The last section of the CD literature involves the application of cyclodextrins in analytical chemistry and diagnostic preparations. The analytical applications of CDs refer mainly to the application of cyclodextrins in gas chromatography, in high-performance liquid chromatography, and in capillary zone electrophoresis, but some papers are dedicated to thin-layer chromatography, to enhancement of UV−vis absorption, luminescence/phosphorescence by CDs, and to increasing the sensitivity of the related analytical methods. Figure 7 illustrates the number of the CD publications in this area. While 10 years ago not a single paper had been published on the application of CDs in capillary electrophoresis alone, in 1997, 195 papers have already been published in this field. For the cyclodextrin producers, this is not a very interesting field because these methods use only milligram quantities for each measurement, but they must be highly purified, free of any UV-absorbing impurities. The number of papers on the gas chromatographic application of CDs seems to have reached a plateau. The HPLC applications keep growing, and the capillary zone electrophoresis application is showing an explosive increase. CDs display unprecedented potential for chiral separation, on a chromatographic scale, of enantiomers. Apparently, it is difficult to find a separation problem on analytical scale which could not be solved by using the appropriate CD.

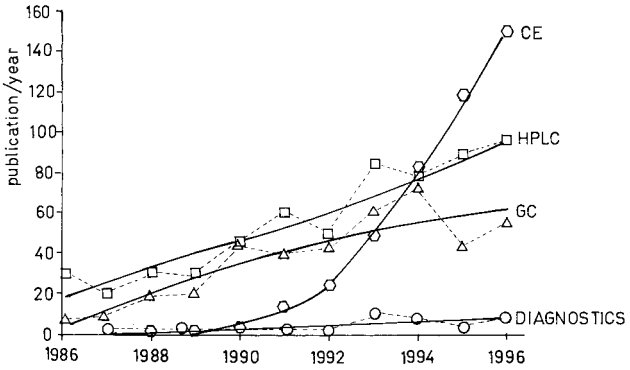

Figure 7. Number of publications per year on application of CDs in analytical chemistry (chromatography, capillary electrophoresis, diagnostics) in 1986−1996 (from *Cyclodextrin News*, ref 28).

Apparently, also, the diagnostics producers have not yet discovered this field, which certainly will open quite a lot of new possibilities, besides improving the actually available diagnostic kits. During the last 15 years, several detailed monographs[32−38] have been published on CDs and their actual/potential applications.

4. Future Trends

4.1. Research

The steady increase of the CD literature is illustrated by the heading of the monthly published *Cyclodextrin News* (Figure 8).[28]

The number of publications in any scientific research area usually consists of three stages. In the first stage, the discovery period, a few sporadic papers are published on the subject. This is followed by a logarithmic increase (second stage) which, after passing an inflection point, turns to a plateau. Finally, in the third stage, the number of publications begins to drop back, at which point the field is exhausted. For the researchers not much remained

CYCLODEXTRIN NEWS

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150 ABSTRACTS IN THIS MONTH!

Total number of publications, abstracted by CD-NEWS up to now:

1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998
379	445	522	693	771	885	924	1168	1033	1370	1458	1585	929	1

Figure 8. The number of CD-related abstracts in *Cyclodextrin News*, from 1985 onward.

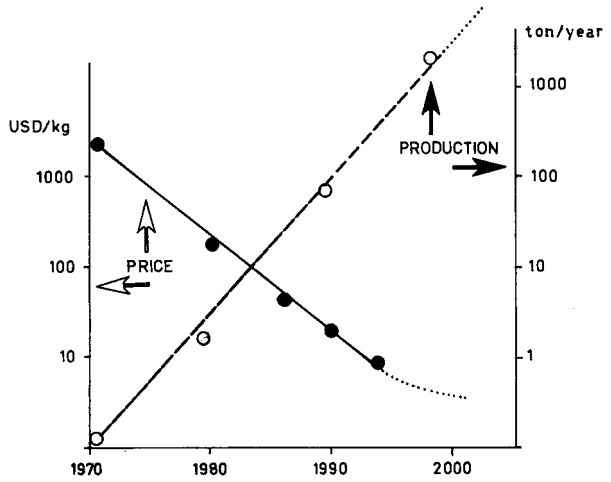

Figure 9. Schematic representation of the correlation between amount of produced β-CD and its price during the last 27 years.

to be discovered or published. As is seen in Figure 8, the CD project has not yet reached the plateau. The leveling-off period is not expected within the coming 5 to 6 years.

4.2. CD Production

Notwithstanding that in the coming decade hundreds of publications will deal with new CTG-ase enzyme producing microorganisms and with enzymatic conversion of starch to CDs, the CD-producing technologies actually used are well established. They will be, of course, continuously optimized, but a fundamentally new technology, a dramatic drop in the production cost is not expected.

The correlation between price and amount of marketed β-CD is illustrated schematically by Figure 9. In 1998 the price of β-CD will reach the acceptable level, even for the most raw material price sensitive industries, like the pesticide industry. Therefore, in the coming decade, a steady increase in the cyclodextrin market is predicted, not only for β-CD, but also for α- and γ-CD. The price of α- and γ-CD will always remain higher than that of the β-CD, partly because of their lower yield (higher solubility), and partly because of the lower volume of their production.

4.3. CD Derivatives

Intensive research is expected in the area of chemical and enzymic modification of CDs. Considering that CDs contain 18 (α-CD), 21 (β-CD) or 24 (γ-CD) substitutable hydroxyl groups, the number of possible derivatives is unlimited. By 1997, the syntheses of more than 1500 derivatives have been published. The known derivatives might be classified according to their substituents, their polarity, size, etc. For practical purposes they can be classified as follows: carriers (solubilizers, stabilizers) for biologically active substances; enzyme models; separating agents (for chromatography or batch processes); and catalysts and additives (as detergents, viscosity modifiers, etc.).

The majority of the reported CD derivatives will never have any utilization because they involve complicated synthesis, resulting in expensive products. Even when they could be used for some of the above-mentioned purposes, the cost/benefit ratio precludes their production and utilization. An industrially produced and marketed CD derivative has to be produced by a simple, possibly "one-pot" reaction and must be nontoxic, when used as recommended; have an acceptable price; retain its complex-forming capacity; and possess particularly advantageous properties for some specific application.

Industrially, in ton amounts, the following CDs are actually produced: methylated CDs (RAMEB = randomly methylated β-CD); hydroxyalkylated CDs (hydroxypropyl-β-CD and hydroxypropyl-γ-CD); acetylated CDs (acetyl-γ-CD); reactive CDs (chlorotriazinyl-β-CD); and branched CDs (glucosyl- and maltosyl-β-CD).

This list is expected to be completed soon by the sulfobutyl-β-CD and eventually by sulfated CDs, as well CD polymers.

Presently (1997) ~100 different CD derivatives are commercially available as fine chemicals, mainly for use in chromatography, in diagnostics, and as intermediaries for further synthesis.

The enzyme models are generally rather complicated molecules, and their performance until now has not shown any dramatic effect. At least for the next

few years, these experiments and enzyme model CD derivatives will remain as a part of fundamental research in enzymology.

To elongate the actual CD cavity, substituents are attached to the primary or secondary side. This elongation may be hydrophilic in which case hydroxyalkyl groups are attached to the ring, or it might be hydrophobic. For example, substituting the primary hydroxyl groups with long fatty acid chains, "medusa" like molecules can be prepared. These molecules behave as detergents while retaining their complex-forming ability. The coming years will decide the utility of these derivatives.

At present, mainly β- and γ-CD, their hydroxypropylated derivatives, acetyl-γ-CD and also, in some specific cases, α-CD can be considered as drug carriers. Only hydroxypropyl-β-CD, sulfobutyl-β-CD, and γ-CD are supported by satisfactory toxicological documentation as parenteral drug carriers (in 1997). None of them is able to solve all of the solubility and stability problems in parenteral drug formulations. The development of 2−3 more such derivatives can be expected in the coming years. The optimum CD derivative (to be used as parenteral drug carrier) should be very soluble in water, cheap, available in high purity, nontoxic, even in high doses, in chronic treatment, characterized by high solubilizing power for various drugs, stable during heat sterilization and storing in aqueous solution, nonreacting with cholesterol and phospholipids (and other cell-membrane components), free of any intrinsic pharmacological effect, and biodegradable in the circulation and eliminated as small molecular metabolites.

This ideal CD derivative does not yet exist. For organ or receptor targetting, extremely stable and specific affinity-showing CD complexes will be needed.[37] The essence of photodynamic tumor therapy is that such compounds have to be delivered to the tumor tissues, which as a result of irradiation with a strong light become toxic through isomerization or splitting. In this case, upon irradiation, the photosensitive molecules will become toxic just for the tumor cells. For such targeting of the drug, very stable ($10^5-10^7 M^{-1}$) complexes are needed. The duplex homo- or heterodimers of CDS (constructed from identical or two different CDs) form complexes that are more stable by orders of magnitude than the singular CDs. By interconnecting two CDs with appropriate bridges, such duplex-CD derivatives have been prepared. These can form stable complexes with photosensitive porphyrinoid structures and transport them to the target organs. Recently, "antennae"-bearing CDs have been reported. Such oligosaccharide units are attached to CDs, which are receptor specific, because they will be bonded in the living organism on certain specific receptors only. The aim of this effort is to synthesize a receptor-targeting carrier, that has the drug complexed with an antenna bearing duplex-CD which would transport the specific drug to just the target organ.

4.4. Industrial Uses of CDS

The actual or potential uses of CDs in pharmaceuticals, foods, cosmetics, chemical products and technologies are summarized in the relevant chapters of this review, as well as in some other recent CD monographs,[33−38] and confirm, unanimuously, the correctness of the prognosticated steady increase of the CD market for the coming decade. While a series of CD-containing products, or CD-using technologies is widely known in the food, cosmetic, and pharmaceutical industries, for the coming decade, significant new applications are expected from the use of CDs in environmental protection, in biotechnology, and in several industries, like the textile industry.[27] The potentials of CDs in separation technologies, with the exception of analytical chromatographies and electrophoretic techniques, have not yet been exploited and only very preliminary works have been published.

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