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Past, present, and future of cyclodextrin research*

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Abstract: The macrocyclic cyclodextrins (enzymic conversion products of starch) were discovered in 1891, and the structures were elucidated in the mid-1930s. Their industrial significance become obvious in the 1970s, and by now thousand of tons of the three cyclodextrins (α-, β-, and γCD) and of their chemical derivatives and inclusion complexes are produced industrially.

The outer surface of these doughnut-shaped molecules is hydrophilic, but they possess an axial open cavity, which is of hydrophobic character and capable of including other apolar molecules (or their moiety) in case of geometric compatibility. This is the essence of molecular encapsulation by inclusion complex formation.

INTRODUCTION

The properties of cyclodextrins (CDs)—their complexes and derivatives—is a very challenging field for solution chemistry: at first glance, it seems to be surprising that the 7-membered βCD is the less soluble (at 25 °C in water 18 mg/cm^3), the 6-membered αCD is better soluble (140 mg/cm^3), and the 8-membered γCD attains the highest solubility (220 mg/cm^3). A freshly prepared γCD solution—even far below the saturation level—always becomes turbid after a few hours or days. Attaching a few hydrophobic substituents to the poorly soluble βCD, the solubility increases unlimitedly. Attaching methyl groups symmetrically—e.g., in heptakis (2,6-di-*O*-methyl)βCD—this derivative is well soluble in cold water, but with increasing temperature its solubility rapidly decreases, and over 60 °C the substance can be isolated as an nonhygroscopic crystalline substance.

Considering that one, and probably the largest, field of practical utilization of CDs is based on their solubilizing capacity (mainly in the pharmaceutical industry) due attention should be paid to the above-mentioned, and many other CD-related, apparent anomalies by solution chemistry.

HISTORY OF CYCLODEXTRINS

The history or development of an applied research field can be visualized on two levels:

- by the number of publications (including scientific papers, conference abstracts, patents/applications, books) or
- by utilization of the research results in the form of products and technologies, expressed by the amount of products (pieces or tons), the impact of the results on the earlier technologies, and value of the developed technologies—all of these can be expressed by an unambiguous parameter: money.

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While in the case of publications, the measure of their value is expressed by impact factors of the journal, such as where it has been published and/or the number of citations of the given paper, the exactness of both of these factors is ambiguous—only the decision of the market is unambiguous. Is the newly developed product or technology useful or necessary? Does it represent real development? If yes, the value of the invested work can be expressed in measurable parameters (mainly by money), if not it will be forgotten.

A patent application (or a granted patent) (its text will be published and available at any rate) either will be really utilized, or will not be utilized (this last case is true for the majority of the patents), but its essence will be available in most cases also as a scientific full paper or at least as a conference abstract.

Because the publication of a result and its utilization are separated in time frequently by decades (earlier by centuries, but this gap nowadays decreases rapidly), and the utilization depends on the data delivered by the research, the first approach to illustrate the history (or development) of a research field is to analyze the number of relevant publications.

The data for the number of publications are always available and up-to-date, but the only measure for the value of the singular papers (from the last two decades) is the impact factor, which is determined exclusively by the forum of the publication. It is not true, that the high-impact-factor journals publish only high-value, original results. Everybody can find painful explanations by highly reputed professors because their coworkers and Ph.D. students published fully unfounded, phantasm-products. On the other hand, if a scientist from a rarely mentioned Pacific Ocean island held a lecture at a Pacific Region Annual Meeting, and it is really interesting and new, it will be refereed by *Chemical Abstracts*, or if it is a CD-related paper, by *CD News*. In our electronic age, anything that is interesting is available, either electronically or as a photocopy. A paper is available electronically only if it is relatively new, e.g., not older than five years. Earlier voluminous literature is regrettably not available in developing countries, e.g., for the majority of Chinese universities from where nowadays the largest number of new manuscripts are submitted for publication. The majority of these papers contain nothing else as rediscoveries of data, facts, observations, which had been published 20 or 30 years earlier.

The discovery period

The first paper [1] that reported the observation on the formation of some unidentified crystalline substance at fermentation of starch was published in 1891. Villiers, the French author, assumed that this substance is some sort of cellulose, and named it "cellulosine". About 15 years later, an Austrian microbiologist, Franz Schardinger [2], studying those microorganisms which play a role in the deterioration of foods, isolated a microorganism (named *Bacillus macerans*) which produced reproducibly two distinct crystalline substances when cultivated on starch-containing medium. Because most of their properties were similar to the already known partial degradation products of starch, the dextrins, he named them α-, and β-dextrin [3,4]. Of course, their chemical structure was not known, but at that time it was not clear that starch (or cellulose) is a macromolecule, consisting of thousands of glucopyranose units.

Freudenberg and his coworkers elucidated the cyclic structure of these two dextrins only in the second half of the 1930s. These 45 years, from 1891–1936, can be considered the "discovery stage" in the history of CDs (see Fig. 1).

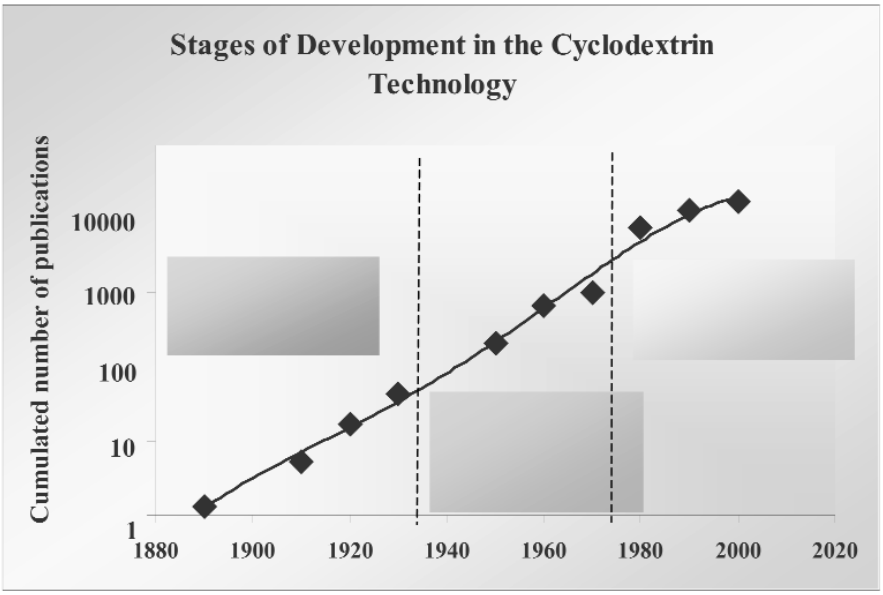

Fig. 1 Number of CD-related publication since 1891.

The "exploratory" period from 1936–1970

At the beginning of the second period, in the 1930s, Freudenberg and his coworkers [5–7], 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. They described the first scheme for the isolation of homogeneous and pure fractions, and in 1936 postulated the cyclic structure of these crystalline dextrins [10]. In 1948–1950, the γ-CD had been discovered and its structure elucidated [11].

With the beginning of the 1950s, two groups led by French [12] and Cramer [13] began to work intensively on the enzymic production of CDs, on fractionating them to pure components, and on characterizing their true chemical and physical properties. French [12] 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 [14] obtained a patent in 1953. In this one-and-a-half page patent, they covered practically 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 [16] and in 1968 by Caesar [17].

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, or 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 CDs 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 CD.

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 CDs, their structure, physical, and chemical properties, as well as their inclusion complex-forming properties had been elucidated. Summarizing the literature available at that time, the conclusions could be condensed into three points:

- Cyclodextrins are very interesting, promising molecules, worthy of further study, particularly because of their apparently industrial possibilities.
- Cyclodextrins are very expensive substances, available only in small amounts as fine chemicals.
- Cyclodextrins are apparently highly toxic, therefore, their utilization for human consumption seems to be questionable.

The "utilization" period: From 1970–onward

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 [18]. From 1984 onward, an International CD Symposium has been held every second year (the 12th one in 2004 in Montpellier) with 120 to 170 lectures and posters summarizing recent results [19–27]. According to *CD News*, the total number of CD-related publications was over 26 000 by the end of 2003 [28] (Fig. 2).

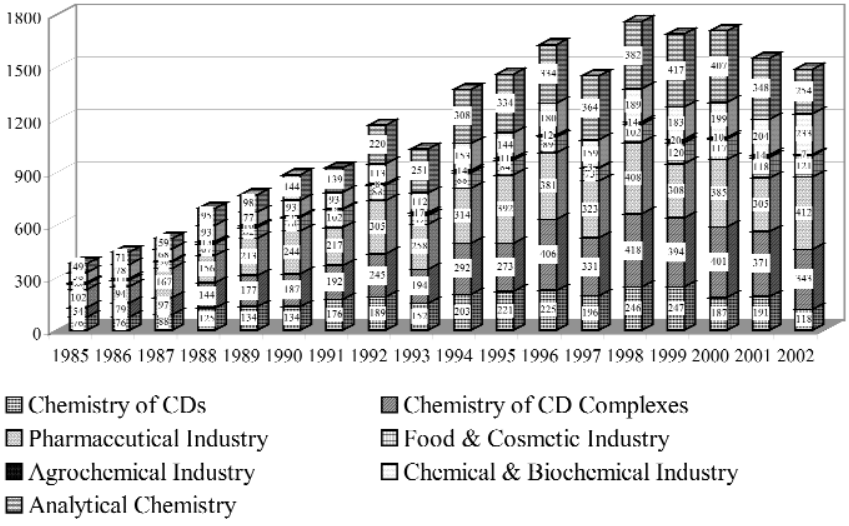

Fig. 2 Number of CD-related publications/year since 1985.

While in 1973 the price of 1 kg of βCD was around 2000 USD, and it was available only as a rare fine chemical, 30 years later, world-wide more than half a dozen companies are producing CDs. Their total output is in excess of 10 000 t/yr, and the price of the key-product, βCD, is only several dollars/kg, depending on quality and delivered quantity (Fig. 3). The α- and γCDs, as well as several derivatives (hydroxypropyl- βCD and γCD, randomly methylated α- and βCD, maltosyl-βCD, acetylated CDs, sulfobutyl βCD, etc.) are produced industrially in 1–100 t amounts. More than 100 other derivatives are available as fine chemicals and used in various chromatographic methods, in diagnostics, or are studied as potential drug carriers, stabilizers, catalysts, etc.

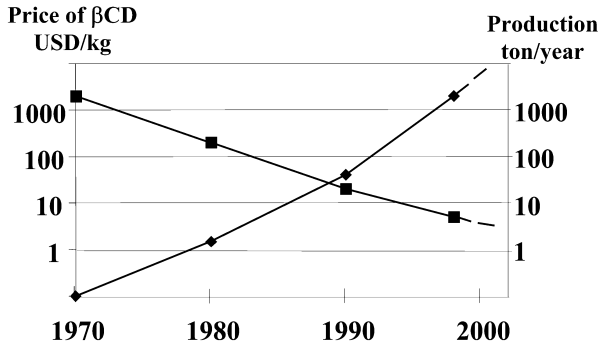

Fig. 3 Correlation between the price (in USD) and produced amount of βCD since 1970.

CYCLODEXTRINS AND THEIR INCLUSION COMPLEXES

Structural features

Cyclodextrins comprise a family of three well-known industrially produced major, and several rare, minor cyclic oligosaccharides. The three major CDs 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, αCD, ACD, C6A) comprises six glucopyranose units, βCD (Schardinger's β-dextrin, cycloheptaglucan, cycloheptaamylose, βCD, BCD, C7A) comprises seven such units and γCD (Schardinger's γ-dextrin, cyclomaltooctaose, cyclooctaglucan, cyclooctaamylose, γCD, GCD, C8A) comprises eight such units (Fig. 4).

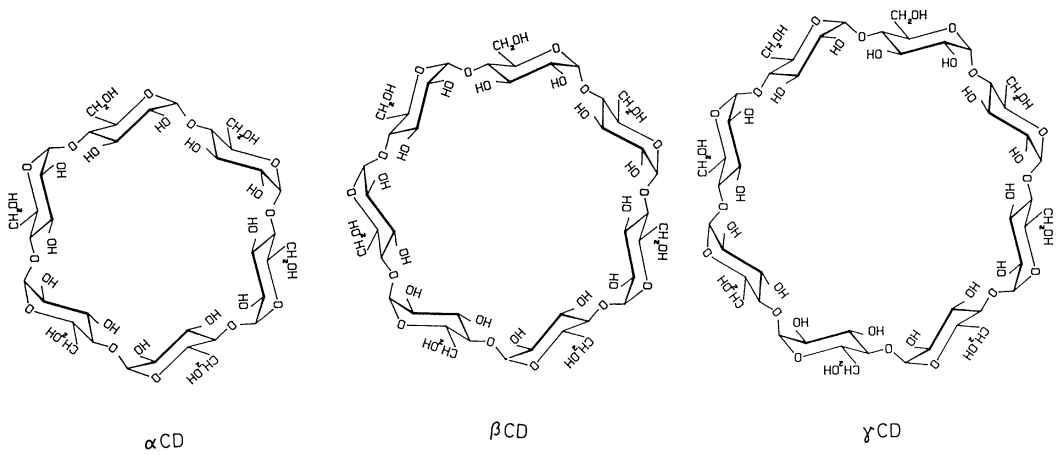

Fig. 4 Chemical structure of α-, β-, γCDs.

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 character.

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 βCD molecule, a complete secondary belt is formed by these H bonds, therefore, the βCD is a rather rigid structure. This intramolecular H-bond formation is probably the explanation for the observation that βCD has the lowest water solubility of all CDs.

The H-bond belt is incomplete in the αCD molecule, because one glucopyranose unit is in a distorted position. Consequently, instead of the six possible H bonds, only four can be established simultaneously. The γCD is a non-coplanar, more flexible structure; therefore, it is the more soluble of the three CDs.

Figure 5 shows a sketch of the characteristic structural features of CDs. On the side where the secondary hydroxyl groups are situated, the cavity is wider than on the other side where free rotation of the primary hydroxyls reduces the effective diameter of the cavity.

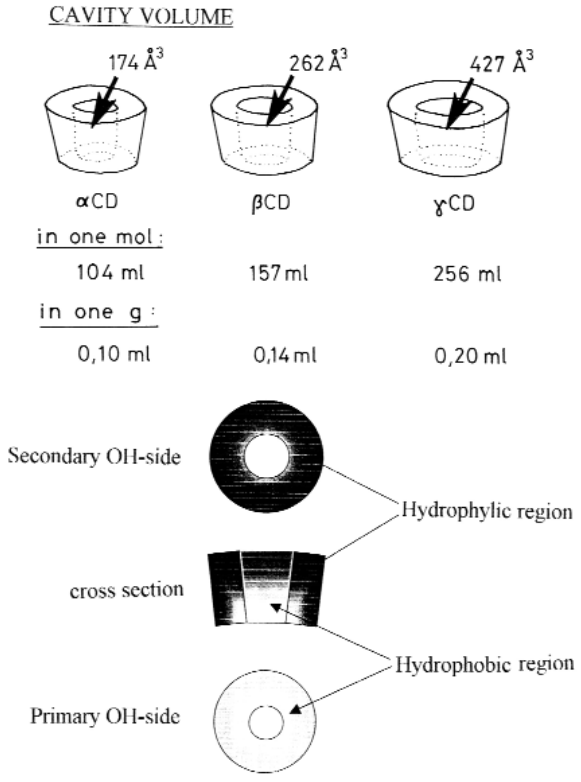

Fig. 5 Dimensions and hydrophilic/hydrophobic regions of the CD molecules.

Cyclodextrins are the most widely used molecules that form host/guest-type inclusion complexes. Although as recently as the 1970s these long-known molecules were merely scientific curiosities, avail-

able only as expensive fine chemicals, by the end of the 20th century they were produced and used industrially in 1000-t amounts.

The spectacular development of CD technology relies on a series of reasons such as:

- they are semi-natural products, produced from a renewable natural material (starch) by a relatively simple enzymic conversion;
- they are produced in 1000 t/yr amounts by environment-friendly technologies;
- their initially high prices have dropped to levels where they become acceptable for most industrial purposes;
- 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;
- any of their toxic effects is of secondary character and can be eliminated by selecting the appropriate CD type or derivative, or mode of application; and
- consequently, CDs can be consumed by humans as ingredients of drugs, foods, or cosmetics.

Cyclodextrin derivatives

In the CDs, every glucopyranose unit has three free hydroxyl groups which differ both in their functions and reactivity. The relative reactivities of C(2) and C(3) secondary, and the C(6) primary hydroxyls depend on the reaction conditions (pH, temperature, reagents). In βCD, 21 hydroxyl groups can be modified substituting the hydrogen atom or the hydroxyl group by a large variety of substituting groups like alkyl-, hydroxyalkyl-, carboxyalkyl-, amino-, thio-, tosyl-, glucosyl-, maltosyl-, etc. groups, thousands of ethers, esters, anhydro- deoxy-, acidic, basic, etc. derivatives can be prepared by chemical or enzymatic reactions. The aim of such derivatizations may be:

- to improve the solubility of the CD derivative (and its complexes);
- to improve the fitting and/or the association between the CD and its guest, with concomitant stabilization of the guest, reducing its reactivity and mobility;
- to attach specific (catalytic) groups to the binding site (e.g., in enzyme modeling); or
- to form insoluble, immobilized CD-containing structures, polymers (e.g., for chromatographic purposes).

From the thousands of CD derivatives described in hundreds of scientific papers and patents, only a few can be taken into consideration for industrial-scale synthesis and utilization. Complicated multistep reactions (using expensive, toxic, environment-polluting reagents) and purification of the products by chromatography are feasible to prepare derivatives only on a laboratory scale. To produce tons, at an acceptable price only about a dozen of the known CD derivatives can be taken into consideration.

Among industrially produced, standardized, and available (even in ton amounts) βCD-derivatives, the most important ones are the heterogeneous, amorphous, highly water-soluble methylated βCDs and 2-hydroxypropylated βCDs (Fig. 6). Due to their heterogeneity, these products cannot be crystallized, which is an important advantage (e.g., at producing liquid drug formulations). It is much more important, however, that these derivatives cannot form crystalline cholesterol complexes, because the unmodified βCD has a particularly high affinity to cholesterol. If administered parenterally, it is not metabolized in the organism, but forms insoluble cholesterol complex crystals in the kidneys, resulting in nephrotoxicity.

A methylated βCD is more hydrophobic than the βCD itself, therefore, it forms a more stable (but soluble) complex with cholesterol. Its affinity to cholesterol is so strong that it extracts cholesterol from the blood cell membranes, resulting in hemolysis already in around 1 mg/cm^3 concentration [32,34].

A particular methylated βCD, the heptakis (2,6-di-*O*-methyl-βCD, called DIMEB) is a crystalline product. It is extremely soluble in cold water, but insoluble in hot water; therefore, its purification, and

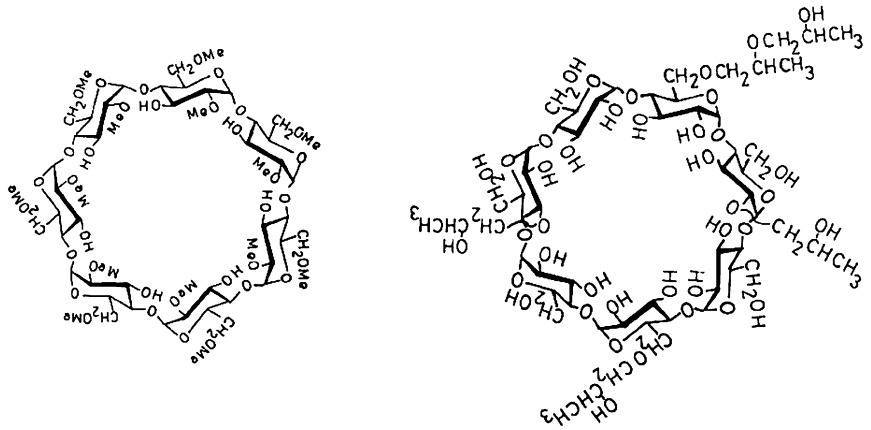

Fig. 6 Structure of the heptakis (2,6-*O*-dimethyl)-β-cyclodextrin and of a hydroxypropyl βCD of DS~4.

also the isolation of its complexes, is technically very simple. Up to now, no better solubilizer has been found among the CDs. It is available in more than 95 % isomeric purity for injectable drug formulation, but for widespread industrial application the cheaper randomly methylated βCD (called RAMEB) is produced and marketed.

The heptakis-(sulfobutyl)-βCD is very soluble in water, noncrystallizable, and even at extremely high doses seems to be free from any toxic side-effects. It can be used as chiral separating agent in capillary zone electrophoresis, but the aim of the intensive research is to develop it as a parenteral drug carrier, for preparation of aqueous injectable solutions of poorly soluble drugs.

The CD-sulfates possess many similar properties to heparin without its anticoagulating effect. Apparently, they can reduce the blood supply of tumor tissues through inhibiting the formation of new arteries.

Cyclodextrin inclusion complexes

In an aqueous solution, the slightly apolar cyclodextrin cavity is occupied by water molecules that are energetically unfavored (polar–apolar interaction), and therefore can be readily substituted by appropriate "guest molecules", which are less polar than water (Fig. 7). The dissolved cyclodextrin is the "host" molecule, and part of 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 CD molecules

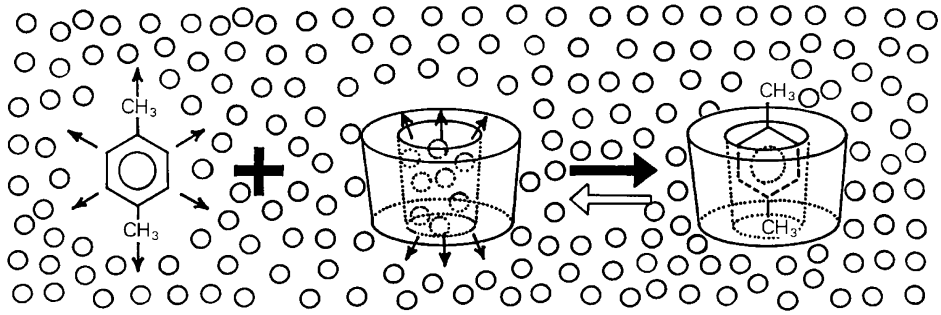

Fig. 7 Schematic representation of the association–dissolation of the host (cyclodextrin) and guest (*p*-xylene). The formed guest/host inclusion complex can be isolated as a microcrystalline powder.

contain one or more entrapped "guest" molecules. Most frequently, the host:guest ratio is 1:1. This is the essence of "molecular encapsulation" (Fig. 7).

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

The most important primary consequences of stirring a poorly soluble guest with an aqueous CD solution are as follows:

- The concentration of the guest in the dissolved phase increases significantly, while the concentration of the dissolved free CD decreases. This latter point is not always true, because ionized guests or 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 necessarily 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 the 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 proven in solution, but the isolated product is nothing other than a very fine dispersion of the CD and the guest.

UTILIZATION OF CYCLODEXTRINS

The most thoroughly studied field of application of CDs is the pharmaceutical industry, more exactly, the use of CDs in drug formulations [29] (Table 1).

Table 1 Approved and marketed drug/CD formulations (2003).

Drug/cyclodextrin	Trade name	Indication	Formulation	Company/country
PGE_2/βCD	**Prostarmon E**	Induction of labor	Sublingual tablet	Ono, Japan
PGE_1/αCD	**Prostavasin**	Chronic arterial	Intraarterial inj.	Ono, Japan
20 μg/amp.	**Edex**	occlusive disease, erectile disfunction	Intracavern inj.	Schwarz, Germany
PGE_1/αCD 500 μg/amp.	**Prostandin 500**	Controlled hypotension during surgery	Infusion	Ono, Japan
OP-1206/γCD	**Opalmon**	Buerger's disease	Tablet	Ono, Japan
Piroxicam/βCD	**Cicladol, Brexin**	Anti-inflammatory, analgesic	Tablet, sachet, and suppository	Masterpharma, Chiesi, Italy
Garlic oil/βCD	**Xund, Tegra, Allidex, Garlessence**	Antiartherosclerotic	Dragees	Bipharm, Hermes, Germany Pharmafontana, H, CTD, USA
Benexate/βCD	**Ulgut, Lonmiel**	Antiulcerant	Capsules	Teikoku, Japan Shionogi, Japan
Iodine/βCD	**Mena-Gargle**	Throat disinfectant	Gargling	Kyushin, Japan
Dexamethasone, Glyteer/βCD	**Glymesason**	Analgesic, anti-inflammatory	Ointment	Fujinaga, Japan
Nitroglycerin/βCD	**Nitropen**	Coronary dilator	Sublingual tablet	Nippon Kayaku, Japan
Cefotiam-hexetil/αCD	**Pansporin T**	Antibiotics	Tablet	Takeda, Japan
Cephalosporin (ME 1207)/βCD	**Meiact**	Antibiotics	Tablet	Meiji Seika, Japan
Tiaprofenic acid/βCD	**Surgamyl**	Analgesic	Tablet	Roussel-Maestrelli, Italy
Diphenhydramine.HCl chlortheophylline+βCD	**Stada-Travel**	Travel sickness	Chewing tablet	Stada, Germany
Chlordiazepoxide/βCD	**Transillium**	Tranquilizer	Tablet	Gador, Argentina
Piroxicam/βCD	**Flogene**	Anti-inflammatory, analgesic for pediatric use	Liquid	Aché, Brasil
Hydrocortisone/HPβCD	**Dexacort**	Mouth wash against aphta, gingivitis, etc.	Liquid	Island
Itraconazole/HPβCD	**Sporanox**	Esophageal candidiosis	Liquid	Janssen, Belgium
Cloramphenicol/ methyl βCD	**Clorocil**	Eye drop, antibiotic agent	Liquid	Oftalder, Portugal
Cisapride/βCD	**Coordinax Prepulsid**	Gastrointestinal mobility stimulant	Rectal suppository	Janssen, Belgium
Nimesulide/βCD	**Mesulid Fast Nimedex**	Nonsteroid anti-inflammatory	Oral sachet	Novartis (LPB), Italy
Nicotine/βCD	**Nicorette Nicogum**		Sublingual tablet chewing gum	Pharmacia Upjohn, Sveden, Pierre Fabre, France
Dextromethorphan/βCD	**Rynathisol**	Antitussive		Synthelabo, Italy
Omeprazole/βCD	**Omebeta**	Proton pump	Tablet inhibitor	Betapharm, Germany
Mitomycin/HPβCD	**MitoExtra Mitozytrex**	Anti-inflammatory	Infusion	Novartis, Switzerland
Diclofenac Na/HPγCD	**Voltaren ophtha**	Nonsteroid anti-inflammatory	Eye drop	Novartis, Switzerland
Cetirizine/βCD	**Cetirizin**	Antiallergic		Losan Pharma, Germany
Ziprasidone mesylate/ sulphobutyl βCD	**Zeldox, Geodon**	Antischizophenic	i.m. inj.	Pfizer, USA
Voriconazole/ sulfobutyl-βCD	**VFEND®**	Antimycotic	i.v. inj.	Pfizer, USA

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Table 1 (*Continued*).

Drug/cyclodextrin	Trade name	Indication	Formulation	Company/country
Tc-99 Teboroxime/HPγCD	**Cardiotec**	Radioactive imaging agent	i.v. inj.	Bracco, USA
17-β-Estradiol/MeβCD	**Aerodiol**	Nasal spray	Liquid	Servier, France

The primary consequences of the CD complexation of drugs are as follows:

- The **rate of dissolution** and the **solubility limit** increase (frequently by a factor of 10^1 to 10^3), resulting in an accelerated and significantly improved bioavailability. It means a reduction of T_{max} (time to reach the blood level peak after administration of the drug), an increase of c_{max} (the attained highest blood level of the drug), and AUC (area under curve expressed as microgram or nanogram multiplied by the hours and divided by the blood volume) (Fig. 8). For example, these parameters for granulated nimesulide and granulated nimesulide/β-cyclodextrin complex (marketed as Mesulide Fast) are as follows: the T_{max} values are 2.17 vs. 1.00 h, the c_{max} values are 4.69 vs. 4.95 μg.h/cm^3, and the $AUC_{0–24\ h}$ 17.32 vs. 38.42 μg.h/cm^3 [38].

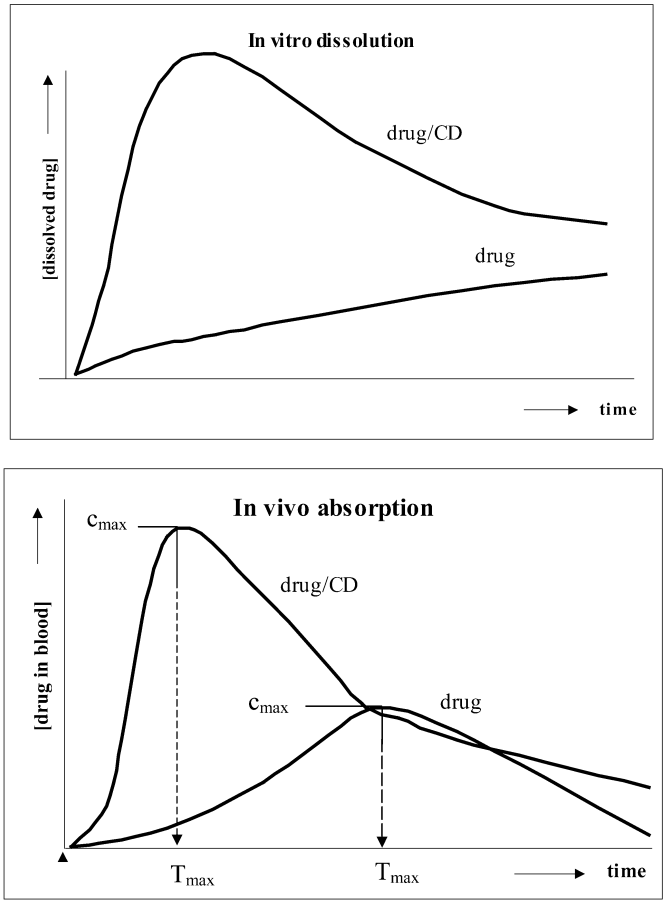

Fig. 8 In vitro dissolution (above) and in vivo absorption of a drug or its CD complex in function of time. The CD complexed drug attains a higher blood level peak (c_{max}) in a shorter time after administration (T_{max}) than the noncomplexed drug. The total bioavailability of the drug is represented by the AUC values, μg.h/cm^3.

- A similar successful product is the piroxicam/β-cyclodextrin, marketed in many countries as Brexin, Brexidol, Cicladol, etc. [39]
- The solubility of Itraconazol is so poor that to prepare aqueous **injectable** solution is hopeless. Its formulation with hydroxypropyl βCD, both a liquid oral formulation as well as injectable solutions, is produced and marketed as Sporanox.
- The **astringent, irritating effect** of nicotine excludes its direct consumption (e.g., in the form of a sublingual tablet or a chewing gum), but in the form of nicotine/βCD complex the mucous membrane (the taste buds in the oral cavity) cannot get into direct contact with nicotine, because it is enwrapped molecularly into the CD capsules. This is the essence of the sublingual tablet for smoking cessation: Nicorette Microtab contains the nicotine in the form of β-cyclodextrin complex.
- Many compounds, like benzaldehyde, cinnamaldehyde, lipid-soluble vitamins, and aroma substances, are prone to rapid **loss**, mainly from solid formulations, **through oxidation, volatilization, and polymerization**. Powder aroma formulations, which contain CD-complexed flavor components, are produced and marketed (e.g., lemon peel oil is fully destroyed by atmospheric oxygen within two days when mixed with any excipient powder, but remains fully stable without any extra protection for years, when complexed with βCD). The flavor will be released only when dissolved in water or saliva, etc. Such CD-based powder aromas are produced and marketed in countries like France, Japan, Hungary, etc.
- Such ingredients as garlic oil (the active component of a popular paramedical product in several countries) are **very bad smelling** and lose their active ingredient content rapidly through disproportionation of the allylsulfides to allylpolysulfides, decomposition of the ajoen to inactive compounds, and by volatilization. Cyclodextrin complexation is the perfect solution of the problem. The first cyclodextrin-containing drug that got approval form the German health authorities was a garlic oil/βCD complex-containing tablet, marketed under the names Xund and Tegra.
- The dextrametorphan bromide is **utterly bitter** (a typically pediatric drug, i.e., administrable only as a "spoon medicine", as a solution, or in suspension). Its very bitter taste can be reduced to a level, which then can be covered by the usual taste-masking sweetening-flavoring compositions. Cetirizine is a bitter anti-allergic drug. When formulated with βCD, the masking of the bitter taste is so successful that a chewable tablet can be produced from it. Ibuprofen (also a bitter drug) in CD-complexed form is appropriate for production of a sparkling tablet (or powder in sachet) formulation, which is devoid of the bitter taste.
- A drug that is typically difficult to formulate, for example, the **very oxygen-sensitive, poorly soluble** unsaturated cyclic hydroxy fatty-acid derivative prostaglandin E_1. Its dose is only 20 μg/vial; after dissolving, it is injected or further diluted for infusion. One freeze-dried vial of the marketed product (Prostavasin, Edex, Viridal) contains besides the 20 μg PGE_1, also 646 μg αCD, which stabilizes and solubilizes the prostaglandin. The sublingual Prostarmon tablet contains 0.5 mg prostaglandin E_2, complexed with βCD.

Table 1 illustrates the steadily increasing use of CDs in the drug formulation.

Less than 10 % of all produced CDs and CD derivatives are consumed by the pharmaindustry. The largest CD consumers are the food and the cosmetic industry. In cosmetic products, CDs are used to: solubilize and stabilize the sensitive components; stabilize emulsions; improve the absorption of active components onto the skin; reduce or eliminate the bad smells of certain components; and reduce the loss of the active components through volatilization, rapid oxidation, destruction by light, etc. In household and toiletry products, the deodorizing capacity of CDs is utilized; in the food industry, the stable flavor powders and the stabilization of water-in-oil emulsions have to be mentioned. For production of low-cholesterol butter, βCD is used to remove the cholesterol from butter.

In the textile industry, a small revolution is expected in the near future. CDs have various possibilities in this industry, but the blockbuster will be the chemical binding of CDs onto the surface of nat-

ural and synthetic fibers. The immobilized CDs can bind volatile molecules like the unpleasant components of sweat or cigarette smoke, etc.; or, when previously charged, the slow release of fragrances, insect repellents, or even drugs (for transdermal delivery) will be possible by wearing bonded CD-containing garments.

In the chemical industry, the number of examples for application of CDs rapidly increases. For example, for conservation of wood products, water-insoluble fungicides have to be impregnated into the wood structures (door and window frames, etc.) Earlier, this was possible only by dissolving these water-insoluble fungicides in organic solvents, now it is possible to use simply aqueous cyclodextrin solutions for this purpose. To reduce the high viscosity of polyurethane thickening agent containing emulsion-type coatings (to facilitate the spraying), CDs are very appropriate, and by adding a small amount of very stable complex-forming detergents just before the spraying, the high viscosity can be restored within minutes.

The pernitro γCD and particularly its complex with nitramine is an excellent missile propellant. CDs can be used in hydrocarbon-polluted soil remediation: the CD mobilizes the insoluble polyaromatic hydrocarbons, bringing them into the aqueous phase, where the soil microorganisms can rapidly metabolize them. CDs, incorporated into packaging plastic films, strongly reduce the loss of the aroma substances by pervaporation. Incorporating CD-complexed fungicides into the packaging films, the fungicides retain their conserving capacity, but cannot penetrate into the enwrapped food (e.g., cheese). This is the way to elongate the shelf-life (e.g., of hard cheeses).

Insecticides, herbicides, fungicides, etc. can be complexed with similar results as in the case of drugs, the dose of environmental polluting synthetic agrochemicals could be significantly reduced when complexed by cyclodextrins, and/or their effectiveness could be enhanced. CDs have wide fields of utilization in sensors, in diagnostic kits, and in analytical chemistry, particularly in the chromatographic techniques.

The majority of biotechnology processes means an enzyme-catalyzed transformation of a substrate in aqueous media. The main difficulties that used to arise are the following:

- the substrate is hydrophobic, and sparingly (or hardly) soluble in water;
- the enzyme or the enzyme-producing microbial cells are sensitive to the toxic effects of the substrate or to inhibitors that can even be the product of the transformation;
- the substrate or the product is unstable under the conditions of the enzymatic transformation; and
- isolation of the product from the very heterogeneous system is difficult.

CDs and their derivatives enhance the solubility of complexed substrates in aqueous media and reduce their toxicity, but they do not damage the microbial cells or the enzymes. The enzymatic conversion of lipophilic substrates can be intensified (accelerated, or performed at higher substrate concentrations), both in industrial processes and in diagnostic reagents. The yield of product-inhibited fermentation can be improved, organic toxic compounds are tolerated and metabolized by microbial cells at higher concentrations, and compounds in small amounts can be isolated simply and economically from complicated mixtures.

Examples illustrate the rapidly growing and promising uses of CDs in various operations: the intensification of the conversion of hydrocortisone to prednisolone; the improvement in the yield of fermentation of lankacidine and podophyllotoxin; the stereoselective reduction of benzaldehyde to L-phenylacetyl carbinol; and the reduction in toxicity of vanillin to yeast, or organic toxic substances to detoxificating microorganisms. In the presence of an appropriate CD derivative (e.g., 2,6-dimethyl-βCD), lipid-like inhibitor substances are complexed. The propagation of *Bordatella pertussis* and the production of pertussis toxin therefore increase up to hundred fold. CDs and their fatty-acid complexes can substitute for mammalian serum in tissue cultures.

Until recently, the leprae bacillus (*Mycobacterium leprae*) was considered to be uncultivable under in vitro conditions. The most important energy source for this bacterium is palmitic (or stearic) acid which, however, cannot penetrate through the thick strongly hydrophilic shell of the myco-

bacterium. On solubilizing the fatty acids (or fatty alcohols) with dimethyl-βCD, however, the Mycobacterium can be cultivated in vitro, on synthetic media. This discovery will facilitate the screening of drugs against similar difficultly cultivable microorganisms.

CYCLODEXTRIN LITERATURE

The CD literature delivers an excellent example of the problem, which at the beginning of the third millennium will represent the major difficulty for a scientist: to find, read, and correctly interpret all the literature, which is pertinent to their 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 last 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 were published 15–25 years earlier, but the authors of the new publication did not find, read, or cite the earlier one.

By the end of 2003, the number of CD-related publications is more than 26 000, representing more than 170 000 printed pages. The productivity of many scientists, mainly at universities and academic laboratories, is evaluated by the number of 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 if they are 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 abstracts—that the author was able to find and read, mixing up significant, industrially important observations and products, with unfounded speculations and nonfeasible ideas. While 30 years ago about 4–5 CD papers were published monthly, in 2003 just that many are published daily.

Figure 9 illustrates the classification of the CD papers, according to their subject, abstracted by *CD News* in 2001 [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 two–three new such publications monthly) is rather low. Papers reporting on the preparation of cyclodextrins from various starch sources like potato, sago, various food industry by-products, 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,

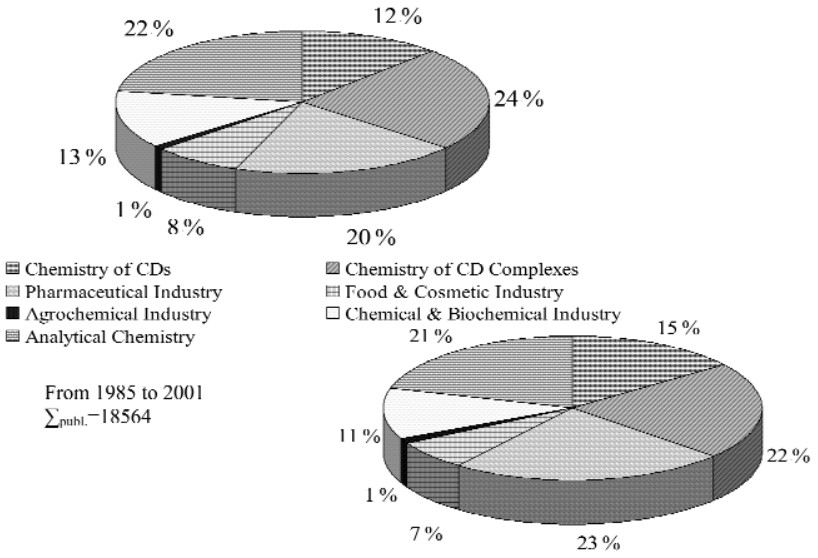

Fig. 9 Distribution of the publications according to the target field between 1985 and 2001 (below) and in the year of 2001 (above).

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 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 enables 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 a slow, but steady development. Nevertheless, the large number (more than 5000) of drug/CD-related papers and patents is a little misleading, because many authors publish the same result 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 haemolysis, or else their synthesis is too expensive. For example, it makes no sense to study a drug/trimethyl βCD 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. The approval process for CD-containing products in this field is much simpler and faster than 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 E_1/αCD complex, but an ampoule contains only 20 μg prostaglandin E_1 and 646 μg αCD. Consequently, less that 1 kg of αCD is needed for the production of one million prostaglandin ampoules. For the production of other successful drug/CD complexes like Brexin (Piroxicam/βCD complex), which is marketed in many countries of the world, not more than 40–50 ton/year of βCD is needed. However, a single toiletry product, like a fragrance tissue, or a deodorant spray for furniture, curtains, or carpets, which needs no health authority approval because it is not consumed by humans and is used only in laundries, requires hundred of tons of βCD or hydroxypropyl-βCD every year. Many tons of βCD are used, for example, for the production of low-cholesterol butter, where the βCD 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 recently the price of even the cheapest technical-quality βCD was simply too high for pesticide formulations. This situation, however, is changed by now, because the price of technical-quality βCD, which is perfectly acceptable for the pesticide formulation industry, dropped to less than USD 4/kg. 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 like lipids, steroids, etc., the possibility of very rewarding CD applications is already known.

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 (HPLC), 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. While in 1986, not a single paper had been published on the application of CDs in capillary electrophoresis, by 1996, 150 papers have already been published in this field. For 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 the analytical scale which could not be solved by using the appropriate CD.

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 25 years, several detailed monographs have been published on CDs and their actual/potential applications [30–36].

FUTURE TRENDS

Research

The steady increase of the CD literature is illustrated by Figs. 1 and 2 [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 into a plateau. Finally, in the third stage, the number of publications begins to drop back, at which point the field be-

gins to be exhausted. For the researchers, not much remains to be discovered or published. As seen in Fig. 9, the CD project is approaching the plateau.

The number of publications will not decrease for a while. Twenty years ago, the number of publications from China was not worth mentioning. Nowadays, nearly half of all CD-related publications are coming from the several hundred Chinese universities. The earlier literature is apparently not accessible for those laboratories since they report on observations and results that were published by European, American, or Japanese laboratories 30 or even more years ago.

The rediscovery is not a rare case, however, even in leading laboratories. For example, the complexation of spironolactone with βCD results not in the spironolactone/βCD complex (except at very low pH values) because the βCD hydrolyzes the thioacetate linkage, and the deacetylated free-SH group containing metabolite can be isolated, as βCD complex. The UV spectrum will not change much, only a careful chromatographic method reveals that beyond complexation a chemical reaction occurred, too.

Not reading the original publication (a patent refereed by *Chemical Abstracts*) or any other pertinent reviews during the last 15 years, 3 laboratories reported again (as an original discovery) the above observation. (Altogether, 56 papers have been dedicated to some aspect of the spironolactone/CD interaction!)

Worldwide, the subject of hundreds of Ph.D. theses is the CD complexation of some drug molecule, which will be also published, therefore, no wonder that 129 publications among them 17 patents/applications are dedicated to such a simple drug as, for example, ibuprofen.

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 actually used CD-producing technologies are well established. They will be, of course, continuously further optimized, but for a fundamentally new technology, a dramatic drop in the production cost is not expected.

The correlation between price and amount of marketed βCD is illustrated schematically by Fig. 3. By 2003, the price of βCD reached 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 βCD, but also for α- and γCD. The price of α- and γCD will always remain higher than that of the βCD, partly because of their lower yield (higher solubility) and partly because of the lower volume of their production.

CD derivatives

Intensive research is expected in the area of chemical and enzymic modification of CDs. Considering that CDs contain 18 (αCD), 21 (βCD), or 24 (γCD) substitutable hydroxyl groups, the number of possible derivatives is unlimited. By 2003, the syntheses of more than 1500 derivatives have been published. The known derivatives might be classified according to their substituents, 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)
- 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 βCD)
- hydroxyalkylated CDs: hydroxypropyl βCD and hydroxypropyl γCD
- acetylated CDs: acetyl γCD
- reactive CDs: chlorotriazinyl βCD
- branched CDs: glucosyl and maltosyl βCD

This list is expected to be expanded soon by the sulfobutyl βCDs and eventually by sulphated CDs, as well CD polymers.

Presently (2003) about 100 different CD derivatives are commercially available as fine chemicals, mainly for use in chromatography, in diagnostics, and as intermediates 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 the fundamental research in enzymology.

To elongate the actual CD cavity, substituents are attached to the primary or secondary side. This elongation may be hydrophylic, 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 γCD, their hydroxypropylated derivatives, acetyl γCD and also, in some specific cases, αCD can be considered as drug carriers. Only hydroxypropyl βCD, sulfobutyl βCD, and γCD are supported by satisfactory toxicological documentation as parenteral drug carriers (in 2003). None of them is able to overcome all of the solubility and stability problems in parenteral drug formulations. The development of two–three 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 inherent pharmacological effect; and
- biodegradable in the circulation and eliminated as small molecular metabolites.

This ideal CD derivative has not yet been discovered. For organ- or receptor-targeting, extremely stable CD complexes of specific affinity will be needed. 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 only one or two different CDs, Fig. 10) 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.

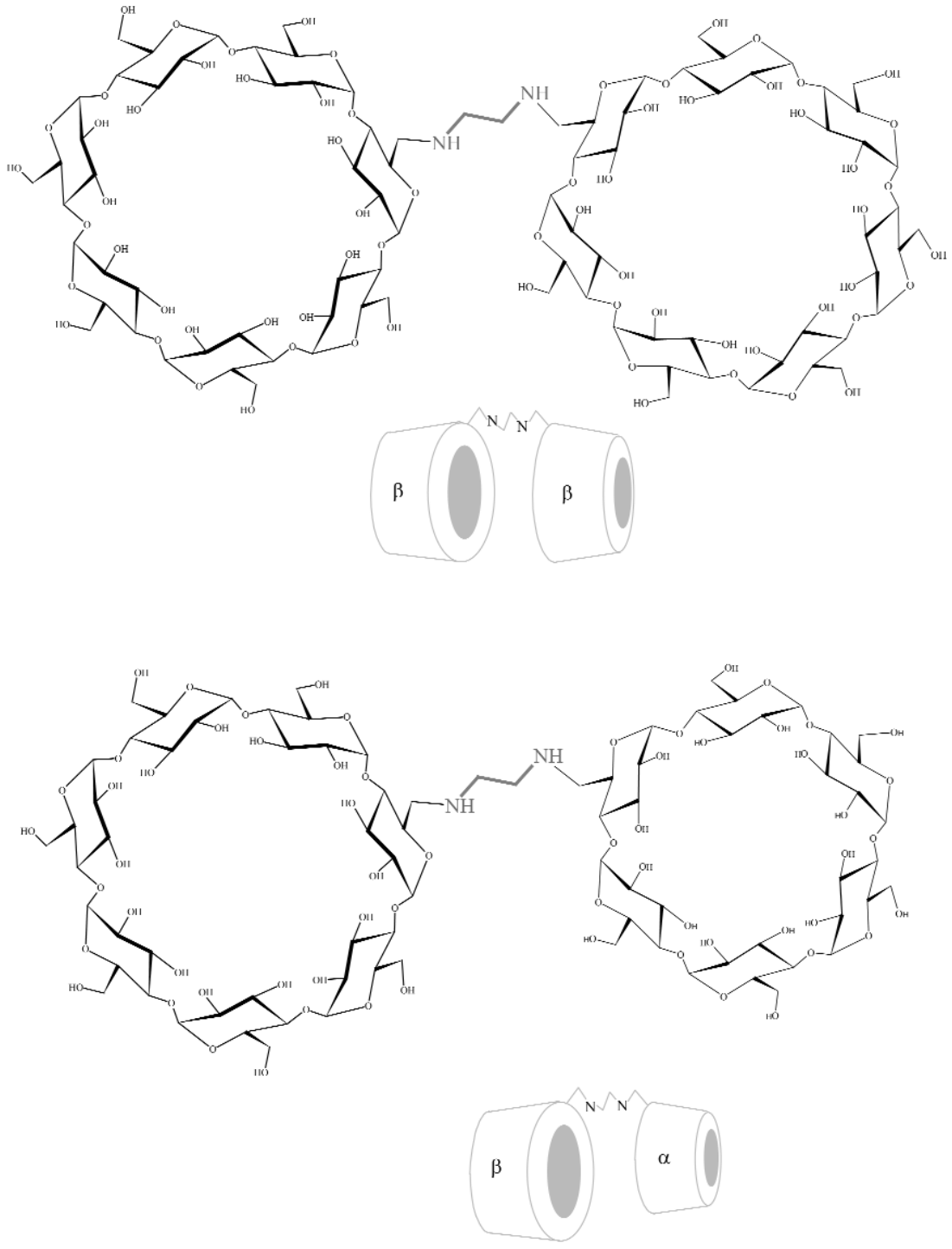

Fig. 10 Structure of a homodimer and of a heterodimer CD.

Receptor-specific oligosaccharide units are attached to CDs, so 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.

Industrial uses of CDs

The actual or potential uses of CDs in pharmaceuticals, foods, cosmetics, and chemical products and technologies are summarized in the relevant reviews, as well as in some recent CD monographs [33–36] and confirm unanimously, the correctness of the forecast of a 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, biotechnology, and in several industries, like the textile industry [37]. The potential of CDs in separation technologies, with the exception of analytical chromatographies and electrophoretic techniques, has not yet been exploited and only very preliminary works have been published.

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