CYCLODEXTRINS IN PHARMACEUTICS, COSMETICS, AND BIOMEDICINE
CYCLODEXTRINS IN PHARMACEUTICS, COSMETICS, AND BIOMEDICINE

Current and Future Industrial Applications

Edited by

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## PART I CYCLODEXTRINS: HISTORY, PROPERTIES, APPLICATIONS, AND CURRENT STATUS

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Discovered toward the end of the nineteenth century, cyclodextrins have attracted the interest of scientists and industries in a variety of sectors. The main reason for this growing interest is the unique structure of the natural cyclodextrins, which enables inclusion of guest molecules in their apolar cavity and masking of the physicochemical properties of the included molecule. The included molecules, mostly hydrophobic, enter a cyclodextrin cavity totally or partially, depending on the size and configuration of the molecule. This book is limited to applications of natural and chemically modified cyclodextrins in the pharmaceutical, biomedical, and cosmetic fields. However, cyclodextrins find use in textile, food, agricultural, and environmental technologies, owing to their unique inclusion complex–forming capability. The relatively low cost of cyclodextrins, being enzymatic degradation products of starch, contributes to their large-scale production as pharmaceutical and cosmetic excipients and resulted recently in the use of a cyclodextrin derivative as an active ingredient in a pharmaceutical product.

Although they were discovered more than a century ago, these “100-year-old spinsters,” as Prof. Dominique Duchene had called them at the 1998 CRS Workshop on Cyclodextrins, have been characterized by an ever-increasing number of publications and patents in the literature, which suggests that cyclodextrins continue to offer new horizons to scientists, with a wide range of possible modifications for adding novel properties to the natural cyclodextrins.

When one reviews the literature on cyclodextrins, the major characteristic of these ying-yang molecules seems to be their solubility enhancement and stability improvement effects on hydrophobic and/or labile active therapeutic or cosmetic ingredients. This effect causes a significant bioavailability enhancement of drug molecules with reduced efficacy due to lower drug absorption and plasma profiles as a result of their low solubility and stability problems, arising from hydrolysis, pH, and photodegradation.

Cyclodextrins produced on a large scale as industrial excipients are used primarily for their solubilizing effect, incorporated in the formulation of analgesic or anesthetic drugs with expected rapid onset. On the other hand, new groups of cyclodextrins are introduced in the pharmaceutical and biomedical fields every day. These exhibit a wide range of properties, including self-assembly, polymerization/condensation, gene delivery, swelling and gelling properties, encapsulation of perfumes and ingredients, and nano- and microencapsulation, which allows cyclodextrins to be actively researched as promising excipients in the nanomedicine, drug delivery, cosmetics, and biomedical fields.

This book consists of two main sections. Part I focuses on the general physicochemical properties of cyclodextrins, such as complexation, as well as drug solubilization and stabilization, which made them come into use in the first place, followed by specific chapters dedicated to various routes of administration, such as oral, mucosal, and skin. This part also covers the most recent findings on the toxicological overview and safety profiles of cyclodextrin derivatives and the regulatory status of cyclodextrins as excipients in the pharmaceutical industry, including the views and applications of regulatory authorities in different parts of the world and corresponding to different markets. The effects of cyclodextrins on the drug release properties of polymeric systems of different types are also discussed in this section, with examples from current literature.

Part II consists of novel and specialized applications of cyclodextrins based on the diversity of modified cyclodextrins. A major group of novel cyclodextrin derivatives are amphiphilic cyclodextrins with different surface charges.
Anionic, nonionic, and cationic amphiphilic cyclodextrins have been reported by research groups, and the self-assembly properties of these new cyclodextrin derivatives give them the capability to form nanoparticles spontaneously in addition to complex-forming properties. Applications of cyclodextrin polymers in gene delivery, peptide and protein delivery, biotechnological applications of cyclodextrins, and novel targeted cyclodextrins destined to carry their load to tumor cells or specific sites such as the colon in complex or conjugated form are also reviewed extensively in this part. Cyclodextrins and their incorporation into polymeric nanoparticles forming new drug delivery systems, cyclodextrin hydrogels, cellular interactions of cyclodextrins, and their relevance in the pharmaceutical and medical fields are discussed as well as the development and marketing story of sugammadex, a pharmaceutical product containing a cyclodextrin derivative as an active molecule. The emergence of cyclodextrins as active molecules rather than smart excipients in therapeutic or cosmetic products seems to be the next step in the discovery and development of cyclodextrin technology.

The goal of this book is to introduce readers of academic or industrial backgrounds to the diverse properties of cyclodextrins, different natural and modified cyclodextrins, and their applications and trends in cyclodextrin research which may be applicable to a variety of industries, such as the pharmaceutical, cosmetic, textile, environmental, and food industries.

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EREM BILENSOY
PART I

CYCLODEXTRINS: HISTORY, PROPERTIES, APPLICATIONS, AND CURRENT STATUS
1. INTRODUCTION

Cyclodextrins (CDs) are molecules of natural origin discovered in 1891 by Villiers. Studied by Schardinger at the beginning of the twentieth century, they became the topic of prominent scientific interest only in the late 1970s, early 1980s [1]. The main value of these oligosaccharides resides in their ring structure and their consequent ability to include guest molecules inside their internal cavity. This is at the origin of many applications: modification of the physicochemical properties of the included molecule (i.e., physical state, stability, solubility, and bioavailability), preparation of conjugates, and linking to various polymers. This results in the use of CDs in many industries, such as agro-food, cosmetology, pharmacy, and chemistry. Presently, the annual average number of articles, book chapters, lectures, and scientific contributions is between 1500 and 2000.

Presented briefly in this chapter are the main cyclodextrins available on the market, and their major characteristics, focusing on their ability to yield inclusion complexes. Also described is the manner in which complexes can be obtained and studied.

2. MAIN CDs AND THEIR ABILITY TO INCLUDE GUEST MOLECULES

2.1. Main CDs

2.1.1. Natural CDs

CDs result from starch degradation by cycloglycosyl transferase amylases (CGTases) produced by various bacilli, among them Bacillus macerans and B. circulans [2]. Depending on the exact reaction conditions, three main CDs can be obtained: α-, β-, and γ-cyclodextrin, comprising six, seven, or eight α(1,4)-linked D(+)-glucopyranose units, respectively [3]. CDs are ring molecules, but due to the lack of free rotation at the level of bonds between glucopyranose units, they are not cylindrical but, rather, toroidal or cone shaped [4]. The primary hydroxyl groups are located on the narrow side; the secondary groups, on the wider side (Fig. 1).

Due to steric factors and tensions in the ring, CDs with fewer than six glucopyranose units cannot exist. On the other hand, although cyclodextrins with 9, 10, 11, 12, or 13 glucopyranose units (δ-, ε-, ζ-, η-, or θ-CD, respectively) have been described, only δ-CD has been well characterized [4]. The largest CDs, those with a helicoidal conformation, are rapidly reduced to smaller products.

The aqueous solubility of CDs is much lower than that of similar acyclic saccharides. This is the consequence of strong binding of CD molecules inside the crystal lattice. Furthermore, for β-CD, with its odd number of glucopyranose units, intramolecular hydrogen bonds appear between hydroxyl groups, preventing hydrogen bond formation with surrounding water molecules and resulting in poor water solubility [4] (Table 1).

The central cavity of CDs, which is composed of glucose residues, is hydrophobic when the external part is hydrophilic because of the presence of hydroxyl groups. In aqueous solution, water molecules inside the CD cavity can easily be replaced by apolar molecules or apolar parts of molecules, leading (reversibly) to an inclusion host–guest complex [5] which can be isolated.

When compared with its free molecular state, the included guest molecule has (apparent) new physicochemical
properties, among which is higher apparent water solubility. This increase in water solubility depends on the CD water solubility, but this parameter is limited compared with linear oligosaccharides. This is one reason that highly water-soluble CD derivatives have been synthesized.

2.1.2. CD Derivatives

CDs’ low aqueous solubility results from hydrogen bonds between hydroxyl groups. Any substitution on the hydroxyl groups, even by hydrophobic moieties, leads to a dramatic increase in water solubility [4]. The different CD derivatives still have the ability to include molecules inside their cavity, but with a different affinity than that of the parent CD. Among the water-soluble CD derivatives most often employed are three classes of modified CDs: methylated, hydroxypropylated (both neutral), and sulfobutylated (negatively charged).

Theoretically, methylation of CDs can occur on either two or three hydroxyl groups per glucopyranose unit. In the first case [dimethyl-cyclodextrins (DM-CDs)] the methylation takes place on all the primary hydroxyl groups (position C₆) and all the secondary hydroxyl groups in position C₂, the secondary hydroxyl groups in position C₁ remaining free. In the second case [trimethyl-cyclodextrins (TM-CDs)] all the hydroxyl groups are substituted, including those in C₃.

Most often, and in the case of β-CD, it is a randomly substituted CD that is used with an average substitution degree (number of substitutions per glucopyranose unit) of 1.8 (e.g., RAMEB, which is an amorphous product). There also exists a very slightly substituted β-CD: Crysmeb, with a substitution degree of 0.5.

Hydroxypropylation occurs in a purely random manner on the primary or secondary hydroxyl groups, leading to an amorphous mixture. Most often, in the case of β-CD, it is 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) that is used; this means that it is a 2-hydroxypropyl moiety that is linked. Because of different producers, the substitution degree has to be mentioned.

There is only one sulfobutylated CD, the β-derivative, with 6.8 substituents per CD (SBE₇m-β-CD). It has about seven negative charges per CD, which are counterbalanced with sodium ions. Usually, a charged group reduces the CD complexation ability, but in the case of SBE₇m-β-CD, it shows high binding properties, due to the significant separation from the CD cavity of the charged sulfonate moieties [5].

2.2. Formation of Inclusion Compounds

2.2.1. Principle

The CD central cavity, composed of glucose residues, is lipophilic and in aqueous solutions can reversibly entrap suitably sized molecules (or parts of molecules) to form an inclusion complex [4]. Formation of an inclusion complex is the result of equilibrium between the free guest and CD molecules and the supramolecules of inclusion:

\[
\text{free CD} + \text{free guest} \leftrightarrow \text{CD/guest (inclusion complex)}
\]

Formation and dissociation of an inclusion complex is governed by a constant \(K\), which may have different names: affinity constant (affinity of the guest molecule for the CD cavity), stability constant (stability of the inclusion complex in a nondissociated form), association constant, or binding constant. The higher the \(K\) value, the more stable the inclusion, and the less dissociation that occurs. The value of \(K\) depends on, among other factors, the size of the CD cavity and that of the guest molecule (or part of the molecule). It also depends on the more-or-less good fitting of the guest molecule inside the CD cavity. As a general rule, the complex is strong when there is size complementarity between the guest and the CD cavity [6]. Depending on their respective size, the guest molecule will enter the CD cavity at the narrow side (primary hydroxyl groups) or at the wide side (secondary hydroxyl groups) (Fig. 2).

2.2.2. Driving Force

The driving force for complex formation has been attributed to many factors, among them the extrusion of water from the cavity; hydrophobic, hydrogen bonding, and electrostatic interactions; induction forces; and London dispersion forces [7]. To better understand the inclusion mechanism, it is important to consider the thermodynamic parameters: the standard free-energy change
Dg), the standard enthalpy change (ΔH), and the standard entropy change (ΔS). Hydrophobic interactions are entropy driven (slightly positive ΔH and large positive ΔS). Van der Waals forces are characterized by negative ΔH and negative ΔS. Compensation (increasing enthalpy related to less negative entropy) is often correlated with water acting as the driving force. In this case, being unable to satisfy their hydrogen-bonding potentials, the enthalpy-rich water molecules from the cycloextrin cavity are released from the cavity and replaced by guest molecules less polar than water, with a simultaneous decrease in the system energy [4].

2.2.3. Different Types of Complexes When speaking of inclusion complexes, it is clear that an apolar molecule, or at least an apolar part of a molecule, is inside the CD cavity. But other complexes can be formed which are not inclusion complexes but in which the guest molecule is linked at the external part of the cycloextrin [8]. Furthermore, depending on the respective size of the guest and host molecules, one guest molecule can interact with one or two (or more) CD (complexes 1 : 1 and 1 : 2) [9], or one or two guest molecules can interact with one CD (complexes 1 : 1 and 2 : 1). For example, Gabelica et al. [6] demonstrated that α-CD forms both inclusion and noninclusion complexes with dicarboxylic acids. The 1 : 1 acid/α-CD complex is mostly (but not totally) an inclusion complex, and the 2 : 1 complex results from the additional formation of a noninclusion complex by interaction of the acid with the 1 : 1 complex.

Loftsson et al. [10, 11] have shown that drug–CD complexes (such as CDs themselves) can self-associate to form aggregates or micelles in aqueous solutions, and that these aggregates can solubilize drugs inside their structures through noninclusion complexation. Furthermore, the less the CDs self-aggregate, the more likely it is that they are involved in interactions with guests [12].

Because of their conformation and size, some guest molecules can be included in one or two CDs (Fig. 3), and depending on the CD size, it is a different part of the guest molecule that can be included (Fig. 4) [13]. Molecules with aliphatic chains fit better into the small α-CD cavity, whereas molecules containing phenyl groups fit better into the larger cavity of either β- or γ-CD [6]. Finally, in solution, multiple inclusion equilibria can coexist (Fig. 5) [14].

2.2.4. Influence of CD Characteristics The nature of CDs has a tremendous influence on their complexation ability. Obviously, the size of a CD is important: It has to be large enough to allow guest entrance but not so large as to be unable to create guest–CD interactions by maintaining the guest molecule inside the cavity, thus preventing a too easy dissociation of the inclusion (low stability constant). The CD derivative substitutions also play a prominent role. In fact, they can either hinder the entrance of the guest or contribute to increasing guest–CD interactions, such as hydrogen bonds between the hydroxyl groups of hydroxylpropyl-CDs and the guest.

In the case of charged SBE7m-β-CD, it is known that placing a charged group on or around a CD usually reduces its complexation ability. This is the consequence of a change in
the CD cavity hydrophobicity and/or a change in the inclusion complexation geometry [15]. However, due to significant separation of the charged substrate moiety from the CD and the possible interaction of some substrates with portions of the butyl moiety, SBE<sub>7m</sub>-β-CD often shows better binding than that of neutral CDs [5].

### 2.2.5. Influence of the Reaction Medium

An inclusion complex between a guest and a CD can be obtained when both entities are in a molecular state. Thus, the complexation efficiency depends on the guest intrinsic solubility ($S_0$) and the complex affinity constant ($K$):

\[
\text{complexation efficiency} = KS_0
\]

An increase in the complexation efficiency can be obtained by an increase in either the guest intrinsic solubility or the complex affinity constant, or by a simultaneous increase in both parameters [16]. In fact, the problem relies most on the guest solubility, the CD solubility most often being much higher than that of the guest. Any substance capable of increasing the guest solubility could be considered to be favorable to the inclusion. But that is not always true.

Organic solvents such as ethanol can increase the guest’s water solubility. However, it competes with the guest for space in the CD cavity, and most often the results are not those expected [17]. In the case of low-water-soluble basic guests, a better method consists of using acids as solubilizers. The enhancement in complexation ability results from both an increase in water solubility of the guest [17] and an increase in the affinity constant due to noncovalent multicomponent (or ion pair) association between the CD, the basic drug, and the acid [18–20]. If ionization of the guest increases its solubility, it can decrease the stability constant, but the increase in solubility remains predominant. Water-soluble polymers can increase the complexation efficiency by an increase in the stability constant [21].

### 3. PREPARATION OF INCLUSION COMPLEXES

The method used to prepare an inclusion complex between a CD and a guest compound has a significant influence on the final product: yield, solubility, and stability of the complex. Most often the nature of the CD to choose depends on the future role of the inclusion. For example, in pharmacy the CD chosen depends on the drug administration route, the fact that the CD is registered in one (or more) of the main pharmacopeia, and the price of the CD. The preparation method has to be adapted to the production level (i.e., industry or laboratory scale) and the objective (i.e., increase in solubility, in stability, etc). Finally, the necessity to add a third or a fourth component for better product solubility has to be considered.

#### 3.1. Preparation Methods

Many methods have been described for the preparation of inclusion complexes. It should, however, be kept in mind that except when the inclusion precipitates spontaneously from the preparation medium, the product obtained is a mixture of three compounds: inclusion complex, empty CD, and free guest. The proportion of inclusion compound is related to the affinity constant of the inclusion complex obtained.

To avoid this drawback, many years ago it was proposed that inclusion compounds be prepared by spontaneous precipitation of the complex from a solution or dispersion of
the guest ingredient dispersed in an aqueous CD solution. The final product had to be washed by organic solvent to eliminate the excess of nonincluded guest. Of course, there are many disadvantages to this technique. To obtain an acceptable yield, it is usually necessary to use a cosolvent of the guest, such as an organic solvent, which unfortunately competes with the guest for inclusion in the CD; and in any case, the yield is very low and the method rather long. Also, and significantly, to be able to precipitate, the complex (and the parent CD) must have low solubility, so the method is restricted to β-CD. The only advantage is that the product obtained is the inclusion only, not a mixture. In fact, it has no industrial use.

Very often, the characteristics of the inclusion complex are compared with those of a physical mixture prepared using the same proportions of guest compound and CD. The hydrophilic character of CDs, comparable to that of saccharides, leads to an increase in the guest compound’s solubility. Furthermore, one cannot exclude the progressive formation of an inclusion complex during a dissolution study. Anyhow, a physical mixture is just a blend and not an inclusion compound.

The co-grinding method [22], in which a physical mixture is submitted to ball-milling in a high-energy vibrational micromill, is interesting because it leads to an almost amorphous product that presents a high level of solubility with fast dissolution. Very similar results have been reported for many products co-ground with cellulose derivatives. In these cases the explanation was the polymer’s role in facilitating amorphization. A similar explanation seems to be logical for co-grinding with CDs.

3.1.1. Co-Evaporation Co-evaporation consists of mixing the guest ingredient in water with the CD (generally, in equimolar amounts) and other components when necessary. The mixing time can be some hours. The solvent can be removed, at a temperature compatible with the stability of the products, in hot air [22] or a vacuum oven [23], or better, to accelerate the process, by evaporation under vacuum in a rotary evaporator [24–26]. The product obtained is more or less crystalline, depending on the nature of the constituents and the exact drying method employed.

3.1.2. Spray-Drying and Freeze-Drying Spray-drying [24, 27, 28] and freeze-drying [24, 25, 27–29] methods are derivatives of the co-evaporation method. To have solutions of good quality adapted to the drying process, they are stirred previously for one or two days or even sonicated. As shown by x-ray diffractograms, the products undergo amorphization during the drying process. Furthermore, the spray-dried product has the appearance of small spheres [24, 27], whereas the freeze-dried product is more amorphous but still has a few crystalline particles [24, 25, 27]. Because of the amorphization, dissolution of both products is very rapid, freeze-drying leading to the fastest dissolution [27].

3.1.3. Kneading Although many processes have been named kneading method, the use of kneading seems to be restricted to the preparation of CD inclusions. Briefly, the guest compound is kneaded together with CD and a small proportion of water or an aqueous solution of ethanol [22, 24, 29], acid [25], or base [27] is progressively added to obtain a slurry. The product may be set aside to equilibrate for 24 or 48 h [24, 27]; or it is kneaded to complete evaporation [22] or dried at 40°C [25] or under vacuum [29]. Amorphization results from the kneading process, but it is still possible to observe some crystals of the original CD and guest compound, as confirmed by x-ray diffractometry and differential scanning calorimetry [24, 27]. Generally, dissolution is better than that of the corresponding physical mixture but slower than that obtained by spray-drying or freeze-drying [27]. However, the results depend on the complex composition: the nature of the CD, the guest compound, and the additives.

3.1.4. Sealed-Heating In the sealed-heating method, CD, guest compound, and additional products, at the desired molar ratio, are placed in a glass container with a very small amount of water. The container is then sealed and kept for 10 to 60 min, or more often 3 h, in an oven at a temperature of 75 to 90°C [22, 26, 30]. Despite the fact that the complex obtained retains some crystallinity [22, 26], its dissolution can be increased dramatically [22].

3.1.5. Supercritical Carbon Dioxide Supercritical fluids are fluids used at temperatures and pressures above their critical value. They are good solvents for nonvolatile and thermolabile compounds. They have gaslike viscosities and diffusivities that promote mass transfer. Their density is similar to that of liquid solvents [31]. In supercritical fluids the diffusivity of dissolved entities is higher than in liquid solvents [32]. This characteristic is favorable to inclusion formation. Carbon dioxide has been widely used since it is safe, inexpensive, nonflammable, and usable in relatively mild processing (supercritical point: 73.8 bar, 31.1°C) [33].

Various types of equipment have been described for the preparation of inclusion complexes. In one type, a physical mixture of guest compound and CD is submitted to supercritical carbon dioxide in a static mode under the required pressure. Following depressurization at the end of the process, the product can be ground and homogenized [31, 34, 35]. Another type of equipment utilizes two main units: one for extraction and one for complex formation. The extraction cell consists of a high-pressure sight gauge packed with alternate layers of glass wool and guest compound. The complex formation unit is loaded with CD. The supercritical
solution of guest compound passes through the CD, and the complex formation cell is isolated and left in static mode. Depressurization is complete within 10 min [36]. The products obtained are less crystalline than the corresponding physical mixtures [22, 26], the intensity of crystallinity depending on the exact preparation conditions (e.g., temperature, pressure). Dissolution is faster than that of the physical mixtures [22, 35], and the bioavailability (liver and kidney tissues) is very good [35].

3.1.6. Microwave Treatment Microwave treatment makes it possible to obtain rapidly high temperatures inside irradiated products. Applied to the preparation of inclusions, it reduces the reaction time significantly [9]. For the preparation of complex, a mixture of guest compound and CD with a minimum amount of solvent is subjected to microwave treatment, most often for 90 s at 60°C (150 W) [9, 37–39]. It also seems possible to subject the physical mixture itself to microwave treatment (500 or 750 W for 5 to 10 min) [30]. The products obtained using this technique show a practically unchanged solid state and are very stable under ambient conditions. Microwave seems more efficient than kneading and co-grinding with respect to dissolution [30].

3.1.7. Choosing a Preparation Method The physicochemical and dissolution properties of inclusion compounds are influenced not only by the constituents—guest compound, CD, ternary or even quaternary systems (organic solvent, acid, base, polymer)—but also by the preparation method. The co-grinding and kneading techniques require only a short operating time and are both potentially industrializable. They could be of great interest for obtaining a limited increase in solubility and dissolution without the necessity of true inclusion [40]. Sealed heating achieves only small amounts of product and is not industrializable. Spray-drying is more expensive, and freeze-drying is both expensive and time consuming, although it seems to be efficient for obtaining true inclusion and amorphization, leading to a rather fast-dissolving product [40]. The use of supercritical carbon dioxide is still experimental. The microwave technique is extremely fast and leads to true inclusions when carried out on products in the presence of liquid [38]. When it is carried out on dried products the unchanged solid state suggests an absence of true inclusion [30]. The absence of true inclusion or a low proportion of true inclusion in the product obtained means that the guest molecule is not protected from the surrounding medium and that its stability will not be improved. However, its solubility can be increased enough for the objective looked at.

3.2. Additives

Various additives can be added either to increase the yield by increasing the affinity constant or to improve the solubility of the inclusion complex. As noted earlier, these additives are water-soluble polymers, and acids, or bases used to form ternary or quaternary systems.

3.2.1. Water-Soluble Polymers The role of water-soluble polymers in the formation and/or solubility of inclusion complexes is multiple and probably depends on both the guest compound and the CD itself. It appears that the role of polymers is not only additive but also synergistic to that of CDs [41, 42]. Polymers can improve the water solubility of the guest compound, a factor favorable to inclusion in CD.

The thermodynamic role of poly(vinylpyrrolidone) (PVP) has been demonstrated. The addition of PVP to the complexation medium of a series of drugs by HP-β-CD results in an increased negative enthalpy change (ΔH°), together with an increased negative entropy change (ΔS°). Thus, the complexation is enhanced (the affinity constant K is increased) upon addition of PVP [41].

When comparing polymers, it has been shown that poly(ethylene glycol) (PEG) has little or no effect on the dissolution of guest/CD complex [42, 43]. This is due to the linearity of the polymer, which can form an inclusion with the CD itself, thus competing with the guest drug. On the other hand, “bulky” polymers such as PVP and hydroxypropyl methylcellulose (HPMC) can form hydrogen bonds with hydroxyl groups of CDs and, more especially, HP-β-CD, leading to a noninclusion complex in which guest molecules can be included in the form of a ternary complex [43]. A very interesting example is that of the inclusion of nabumetone in β-CD, in which the drug is wrapped at both ends by a β-CD molecule, the PVP polymer acting as a bridge between the two β-CD molecules [44].

Numerous polymers have been investigated for their ability to increase the affinity constant and solubility of guest/CD inclusion complexes. The most frequently studied have been PEG [42, 43], poly(vinyl alcohol) (PVA) [45], PVP [41–44, 46–49], HPMC [42, 43, 46], carboxymethylcellulose (CMC) [46], and NaCMC [42, 49]. Unfortunately for future users of CDs and polymers, there is no general conclusion. Depending on the nature of the guest and the CD, the best products are PVP, HPMC, and NaCMC.

The exact preparation method may have some influence on the result. For example, heating the preparation medium (guest, CD, and polymer in aqueous solution) to 120 to 140°C for 20 to 40 min has been claimed to increase the affinity constant and the complexation efficiency of HP-β-CD [16]. Similar results have been obtained by heating at 70°C under sonication for 1 h [21, 43]. The mechanism of this phenomenon, called polymer activation, is not known. Some contradictory results have been published describing the absence of the effects of such treatment [42].

3.2.2. Acids and Bases In the case of acidic or basic ionizable guest compounds, it seems appropriate to adjust
the pH to obtain the higher solubility of the guest, allowing easier inclusion formation and leading to better complexation efficiency [16]. This can be obtained by the addition of a base [23, 50], an acid [50], or by the use of phosphate buffers [43, 49]. Volatile bases (ammonia) or acids (acetic acid) can be removed from the complex during the drying process. A general increase in the guest compound’s apparent solubility is obtained [16, 43, 49, 50]. In any case, the relative increment in solubility with respect to the guest alone obtained by cyclodextrin complexation at the optimal pH can be rather low because of a concomitant reduction in the stability of the complex formed with the ionized guest [49].

In dissolution experiments, precipitation of the guest compound can occur because of a thermodynamically unstable oversaturation of the solution [50]. The high-energy guest/CD complex obtained could lead to enhanced drug delivery through biological membranes and, consequently, enhanced drug bioavailability compared to conventional guest/CD complexes [50]. Tartaric acid has been proved to increase the water solubility and oral bioavailability of vinpocetine when included in either β-CD or SBE-β-CD [51].

A very interesting study has been carried out on the role of maleic, fumaric, and tartaric acids on the inclusion ability of miconazole in β-CD and HP-β-CD [52]. For the ternary complexes obtained, depending on their conformation and/or their structures, the acids can either stabilize or destabilize the complex. In β-CD, maleic acid presents the best conformation for forming a ternary complex. The inclusion yield with this acid is higher than with fumaric. Tartaric acid (L or D) does not affect the inclusion yield; in fact, it has affinity for the CD cavity and can extract miconazole. With HP-β-CD, L-tartaric acid stabilizes the complex, increases the interaction and complexation energies, and promotes miconazole inclusion. L-Tartaric acid does not interact with the imidazole ring of miconazole as maleic and fumaric acid do.

3.2.3. Other Additives The role of various additives, known as solubilizing agents, on the solubility of guest/CD inclusion complex has been investigated.

Anionic organic salts such as sodium acetate and sodium benzoate increase the aqueous solubility of hydrocortisone/β-CD complex [8]. Normally, sodium salicylate forms inclusion complexes with β-CD and should compete with hydrocortisone, resulting in reduced complexation and CD solubilization of hydrocortisone. The favorable effect of sodium salicylate cannot be explained by simple inclusion formation. In the case of sodium acetate, the enhanced solubilization is partially due to increased β-CD and hydrocortisone/β-CD complex solubility. The acetate ions solubilize the hydrocortisone/β-CD microaggregates formed in aqueous solution [8].

For its part, the cationic organic salt benzalkonium chloride has only a limited effect on the hydrocortisone/β-CD complex solubility, possibly because of competing effects between benzalkonium and hydrocortisone for space in the CD cavity [8].

The role of phospholipids (egg phosphatidyl choline and phosphatidylglycerol) on ketoprofen/β-CD and ketoprofen/M-β-CD solubility has also been investigated [30, 39]. The ternary systems obtained have higher solubility, especially ketoprofen/phosphatidylcholine/β-CD. The synergistic effect between cyclodextrins and phospholipids in enhancing drug dissolution is attributed to a combination of the surfactant properties of phospholipids and the wetting and solubilizing power of CDs and/or the possible formation of a multicomponent complex [39].

3.2.4. Quaternary Systems Considering the dissolution enhancements obtained with ternary systems in which are present the guest compound, the CD, and an additive such as polymer, acid, or anionic organic salts, it was logical to investigate the effect of several additives used in quaternary systems. For example, the association sodium acetate–HPMC in the preparation of a quaternary complex of hydrocortisone/β-CD appears to have a better solubilizing effect than that of one or the other additive used alone [8]. Similar results were obtained with the association tartaric acid–PVP added to vinpocetine/SBE-β-CD [51].

4. PHYSICAL STUDIES OF INCLUSION COMPLEXES

When preparing an inclusion complex, the objectives can be of different types: modification of the physical state (liquid or gas transformed into solid), masking of an unpleasant odor or taste, enhancement of solubility, enhancement of stability, and so on. It is not enough to control these effects; it is necessary to know exactly what complex has been obtained. Different physical studies can be carried out in order to check the existence of a true complex, evaluate its stoichiometry, calculate its stability constant, and discover its structure. Different types of studies can be carried out.

4.1. Characterization of the Complex

The objective is to know if there is a complex or just a mixture of guest, CD, and guest compound.

4.1.1. Scanning Electron Microscopy Very often, study of an inclusion complex begins by observing it using scanning electron microscopy (SEM). For the observation, samples are fixed on a brass stub and made electrically conductive by coating with a thin layer of copper [27], gold [23, 24, 40], or gold–palladium alloy [53]. For a good comparison, samples should be observed at the same magnification. It is,
however, difficult to conclude as to the formation of an inclusion because of the morphological change that occurs between the physical mixture and the product obtained. The preparation process generally has a great influence on the characteristics of the product. For example, spray-drying very often leads to small spheroids; co-evaporation or kneading, to large fragments; and freeze-drying, to thin and more or less crystalline particles [24, 25].

4.1.2. Ultraviolet Spectroscopy Because it is a very simple method, the ultraviolet (UV) absorbance spectrum of complexes has been used to study inclusion complexes [23, 29, 54]. Cyclodextrins do not show any significant UV absorbance, so the increase in absorbance observed in a guest/CD solution results from perturbation of the chromophore electrons of the guest by its inclusion in the CD [29].

4.1.3. Circular Dichroism Being symmetrical molecules, CDs have no dichroic activity, but they can modify that of guest molecules by perturbation of the microenvironment polarity resulting from the inclusion [54, 55].

4.1.4. Differential Scanning Calorimetry Differential scanning calorimetry (DSC) analysis is very often carried out on raw materials and products resulting from the complex preparation process [22, 24–27, 29, 30, 40, 53, 55]. In fact, when guest molecules are included in the CD cavity, their melting, boiling, and sublimation points usually shift to a different temperature or disappear within the temperature range at which the CD is decomposed [53]. Generally, the samples are heated in a sealed pan from 25 to 250°C, 300°C, or even 450°C at a rate of 5 or 10°C/min, under nitrogen or air. An empty sealed pan is taken as a reference. CDs, in particular β-CD, contain water molecules inside their cavity. This water is released at the beginning of the temperature increase. In a sealed pan, the presence of vapor can perturb the observation of further thermal accidents, especially if the scanning rate is too fast. To prevent this drawback, some authors prefer to work with pierced pans [24, 25, 30].

In a classical experiment, the melting peak of the guest will disappear or decrease (or shift) by its inclusion in a CD, depending on the proportion or true inclusion and free guest in the product under investigation. However, the disappearance of or decrease in the guest melting peak can result from its amorphization by the preparation process, in particular freeze-drying. Thus, the results have to be interpreted with great care.

4.1.5. Infrared Spectroscopy Infrared (IR) analysis can give interesting information on the products obtained by association of a guest to a CD and is frequently employed [22, 24–27, 29, 30, 40, 53], either as classical IR spectroscopy or as Fourier-transformed infrared spectrometry (FTIR). IR analysis is carried out on the powders included in a KBr disk [29, 53]. FTIR analysis can be performed either on powder samples dispersed in Nujol [22, 26, 30, 40] or directly on the powder samples themselves [24, 27], which technique prevents any transformation of the products. At present the FTIR method is the one most commonly employed. A classical FTIR analysis is performed by application of 16 scans at a resolution of 4 cm⁻¹ over the range 4500–4000 to 600–400 cm⁻¹.

The physical mixture leads to a superposition of the two spectra (i.e., guest, CD) without any change. Normally, in a simple inclusion complexation no new bands should appear, which would be indicative of new chemical bonds in the product obtained corresponding to another type of interaction [27]. On the other hand, inclusion complexation leads to significant changes in the characteristic bands of the guest molecule. For example, the strong reduction or complete disappearance of the characteristic bands is indicative of strong guest–CD interactions and possibly inclusion complexation [24, 53]. A shift of a carbonyl stretching band to higher frequencies with concomitant broadening and decrease in intensity can be attributed to the dissociation of intermolecular hydrogen bonds associated with crystalline molecules and can be observed for complexes obtained by freeze-drying [29].

4.1.6. X-ray Diffractometry Powder x-ray diffractometry (XRD) is used to measure the crystallinity of a product. Even if a change (lost) of crystallinity does not prove the inclusion, it is very frequently employed in the study of inclusion complexes [22, 24–27, 29, 30, 40, 53]. Most often the analysis is carried out with Cu [22, 26, 29, 30, 53] or Co [24, 25, 27, 40] Kα radiation with a voltage of 40 to 45 kV and a current of 35 to 40 mA over the 20 range 2–5°C to 38–70°C.

In an XRD pattern the intensity of diffraction peaks is indicative of the crystalline character of the product. A hollow pattern is characteristic of amorphous products [27]. The relative degree of crystallinity can be calculated as the ratio of the peak height of the sample under investigation to that of the same angle for the reference with the highest intensity [24, 25, 27].

The different cyclodextrins do not exhibit the same crystallinity: β-CD and DM-β-CD are rather crystalline, whereas M-β-CD (Crysmeb), SBE-β-CD, and HP-β-CD exhibit an amorphous character.

Diffractograms of physical mixtures result from a combination of the diffractograms of the components analyzed separately. When a CD has an amorphous character, a decrease in peak intensity can be observed [27].

When studying a prepared inclusion complex, a decrease in crystallinity, shifts in and the disappearance of peaks, the appearance of new diffraction peaks, or a completely diffuse pattern might be related to possible guest amorphization.
and/or complexation [26]. A strong reduction in or the complete disappearance of the guest characteristic peaks can be indicative of strong guest–CD interactions and the possible inclusion complexation of the guest [24] or its molecular dispersion in the CDs [53]. Very often the amorphization observed for the inclusion complex is largely dependent on the preparation method. For example, and depending on the products, co-evaporation and sealed heating lead to rather crystalline profiles [26], whereas kneading or supercritical fluids have a variable effect [26]. Most often, freeze-drying leads to some amorphization [25, 53].

4.1.7. Electrospray Mass Photometry
Electrospray (or electrospray ionization) mass spectrophotometry (ES-MS) is a very powerful method of studying inclusion complexes [5, 54, 56–58]. It is a soft method of ionization for nonvolatile and thermolabile molecules which can hardly induce fragmentation [54]. It can provide evidence of complexation and stoichiometry on the basis of the molecular weights of all vaporized species [56–58]. However, there are still ambiguities in the spectra obtained for supramolecular assemblies: Do the species present in the mass spectra correspond to those present in solution, or do they result from processes that occur under high-vacuum conditions? [58].

4.1.8. Proton Nuclear Magnetic Resonance
Proton nuclear magnetic resonance (1H NMR) spectroscopy is probably the technique that can give the most accurate information about inclusion formation [48, 53, 55, 56, 58, 59]. It can be used to prove the inclusion existence, and to determine its stoichiometry, affinity constant, and structure. Samples are dissolved in D2O [48, 58], D2O/CD3OD [53, 55], or CD3OOD/D2O [56] and the experiment is performed at 300, 400, 500, or 600 MHz. Of course, the product that is investigated is a solution and not the solid complex. Insertion of a guest molecule into a CD cavity results in the modification of 1H NMR frequencies. Major changes in the chemical shift values of the CD protons—more specifically, H3 and H5 located inside the cavity, or H6 on the cavity rim—indicate the formation of an inclusion complex [14, 48, 53, 59]. Guest protons interacting with the CD can be evidenced, and noninclusion complexes can be characterized.

4.2. Stoichiometry and Constant of the Complex
As already mentioned, guest/CD complexes can involve one or more guest molecules for one or more CDs and are characterized by their stability constant. If m guest molecules (G) associate with n CD molecules (CD),

\[ mG + nCD \rightleftharpoons G_m \cdot CD_n \]

\( K_{m,n} \) is the stability constant of the guest–CD complex [60].

4.2.1. Higuchi Phase Solubility Diagram
The phase solubility analysis described by Higuchi and Connors [61] is a very classical investigation carried out to better define the complex type [10, 62]. To obtain the corresponding diagram (Fig. 6), a fixed amount of guest compound is added to a series of CD solutions of increasing concentration with a constant volume. It is necessary to use an excess of guest compound in order to maintain the highest possible thermodynamic activity. These solutions are agitated for several hours (or days) up to equilibrium. After filtration the dissolved guest concentration is measured by an appropriate method. The value obtained corresponds to the guest really dissolved (the intrinsic solubility) plus the guest dissolved in inclusion form; it is the guest apparent solubility [62]. Two types of complexes can be obtained: A (a soluble inclusion complex is formed) and B (an inclusion complex with definite solubility is formed) [63].

In type A, the apparent solubility of the guest increases as a function of CD concentration. Three possible profiles exist: A1, A2, and A3. A1 corresponds to a linear increase in solubility with an increase in CD concentration. A2 corresponds to a positive deviation from linearity (the CD is more effective at high concentrations), and A3 corresponds to a negative deviation (the CD is less effective) [62]. In the A1 case and assuming that the complex is of 1 : 1 type, the stability constant of the complex can be calculated from the slope of the isotherm [63]:

\[ K = \frac{\text{slope}}{S_0(1 - \text{slope})} \]

In the A2 case, indicative of higher-order inclusion complexes, the \( K \) value can be calculated using the iteration method. It is difficult to analyze the diagram quantitatively because this system is associated with factors such as solute–solvent or solute–solute interactions [63]. In type B there is formation of complexes with limited water solubility, which are traditionally observed with β-CD. Two different possible profiles exist: B5 and B7. B5 corresponds first to the formation of a soluble
complex, which increases the total solubility of the guest, but at a particular point of this solubilization process, maximum solubility is achieved, corresponding to the guest intrinsic solubility plus the guest solubilized in inclusion complex form. Additional CD generates additional complex that precipitates, but as long as solid guest remains, dissolution and complexation can occur, maintaining a plateau. When the entire solid guest has been consumed, further addition of CD results in the formation of additional insoluble complex, and the final solubility observed in the system is that of the complex itself [62]. B₁ is similar to B₅ except that the complex is so insoluble that it does not increase the guest apparent solubility. The stability constant of B₅ complexes can be calculated from the slope of the ascending part of the isotherm, using the same equation as for A₅ complexes [63].

4.2.2. Permeation When using artificial membranes permeable to the guest but impermeable to the larger CD, the permeation profile of the guest in the presence of CDs in the donor phase is related not only to its permeation rate but also to the complex stability constant [64, 65]. The relationship between the guest permeation rate and the stability constant of the complex is given by

\[ [G_A] = \frac{[G_0] \{1 - \exp(-AR_t)\}}{2 + K'[CD]} \]

where \( A = k(2 + K[CD])/(1 + K[CD]) \), with \([G_A]\) being the guest concentration in the acceptor phase, \([G_0]\) the concentration in the donor phase at time 0, \([CD]\) the concentration of the free CD in the donor phase, \( k \) the guest permeation rate constant, \( K \) the complex stability constant, and \( t \) the time. Therefore, \( K \) and \( k \) can be calculated by analyzing the guest concentration data in the acceptor phase as a function of time using a nonlinear least-squares method [64, 65]. This method has been employed in the determination of the stability constant of hydrocortisone/HP-β-CD, a 1:1 A₅₁-type complex [11] and to that of flurbiprofen/M-β-CD and flurbiprofen/HP-β-CD [65]. It cannot be used for complexes with β-CD because its poor water solubility does not allow having a high enough CD concentration in the donor phase [65].

4.2.3. Nuclear Magnetic Resonance The association constant can be derived from NMR data using the Benesi–Hildebrand graphical method [66]. This is a graphical approach based on the observation of any parameter \( A \) (provided that it is affected by the interaction process considered) for one of the entities in the presence of a large but variable excess of the other entity, B. Chemical shift differences for a given proton can be used as variables [59, 67]. The equilibrium constant can be written

\[ K([A]_t - [C])[B]_t = [C] \]

[C] is related to the chemical shift difference between the free molecule and the complex by

\[ [C] = \Delta P_{obs}[A]_t \Delta \delta_c^{-1} \]

where \( \Delta \delta_c \) is the chemical shift difference. This leads to

\[ \Delta \delta_{obs} = K[B]_t \Delta \delta_c (1 + K[B]_t)^{-1} \]

The Benesi–Hildebrand graphical method allows to rewrite this equation in the form

\[ (\Delta \delta_{obs})^{-1} = (K \Delta \delta_c)^{-1} ([B]_t)^{-1} + (\Delta \delta_c)^{-1} \]

Plots of \( (\Delta \delta_{obs})^{-1} \) against \( ([B]_t)^{-1} \) are linear. The slope, abscissa, and ordinate intercepts are \( (K \Delta \delta_c)^{-1}, -K, \) and \( (\Delta \delta_c)^{-1} \), respectively [67].

A 1:1 stoichiometry is assumed in the theoretical basis. This method has a number of limitations: the entities should be soluble enough, there is a lack of sensitivity for low concentrations, and the effect of viscosity in the presence of a large excess of one of the entities must be assessed. The accuracy of the method drops rapidly as \( K \) increases [59].

Other methods have been described for obtaining the stability constant from NMR data. For example, the diffusion-ordered spectroscopy (DOSY) technique can be used [68]. The association constant \( K \) for a complex of \( m \)-molecule host (CD, H) and \( n \)-molecule guest (G),

\[ nCD + mG \leftrightarrow C[H_nG_m] \]

could be reduced to

\[ K = \frac{[C]}{[H]^n[G]^m} = \frac{[C]}{([H]_0^n - n[C])^n([G]_0^m - m[C])^m} \]

where \([G]_0\) and \([H]_0\) are the total concentration of the guest and host, and \([G], \; [H], \; \text{and} \; [C]\) are the equilibrium concentrations of the free host (H), free guest (G), and the complex (guest/CD, C). If the mole fraction \( \chi_b \) of the bound entities is known, \( K \) is

\[ K = \frac{\chi_b}{(1 - \chi_b)([H]_0 - \chi_b[G]_0)} \]

The diffusion coefficient observed \( (D_{obs}) \) in the NMR experiment (fast exchange conditions) is the weighted average of the diffusion coefficient of bound \( (D_{bound}) \) and free \( (D_{free}) \) guest:

\[ D_{obs} = \chi D_{bound} + (1 - \chi) D_{free} \]
Accurate determination of the association constant implies that the stoichiometry is known unambiguously [68]. The association constant can also be obtained by using a nonlinear least-squares procedure, resorting to the Levenberg–Marquardt algorithm on the differences observed in the chemical shifts due to the presence of CD [69]. The values are calculated using the protons of the guest that lead to largest chemical shift variations in the presence of increased cyclo-dextrin concentrations [27].

NMR studies are also a tool for determination of the complex stoichiometry. The method is that proposed many years ago by Job [70]. It deals with fast exchange systems and can be applied to any technique provided that a given experimental parameter is different in the free and bound states [59]. This parameter is determined for a series of samples prepared by mixing, to constant total volumes, equimolar solutions of the two interacting entities, the total concentration being kept constant. The ratio between the concentration being kept constant. The ratio between the total and free concentrations of A is

\[
P_{\text{obs}}[A] = P_r[C] + P_f[A]
\]

where \(P_r\) and \(P_f\) are the values of the parameter \(P\) observed in the complexed and free forms of A, \([A]\) and \([A]\) being the total and free concentrations of A. Plotting \(\Delta P_{\text{obs}}[A]\) as a function of \(r\) gives a bell-shaped curve exhibiting a maximum for \(r = 1/1 + n\), allowing direct determination of \(n\). \(\Delta P_{\text{obs}}\) being equal to \(P_{\text{obs}} - P_f\), the general shape of the curve depends only on the difference between the value of the parameter observed in the free and complexed states, and not on \(K\). When all plots show a maximum at \(r = 0.5\), it indicates that the complex formed has a 1:1 stoichiometry; a 1:2 complex should provide a nonsymmetrical plot with a maximum at \(r = 0.33\), a 2:1 complex corresponding to \(r = 0.66\) [59]. This method is very often used for the determination of the complex stoichiometry [14, 48, 71–74]. Interestingly, this method also allows to evidence the simultaneous presence of two complexes of different stoichiometry. This is the case for \(\beta\)-CD/triclosan, for which the maximum of the curve is not at 0.5 (for 1:1 complex) or at 0.66 (for 2:1 complex) but at 0.6, indicating that complexes of both stoichiometries are present in solution simultaneously [75].

4.2.4. Ultraviolet Spectroscopy

UV spectroscopy can be used similarly to NMR for determination of either the complex stoichiometry or its association constant [76, 77]. In this case, it is the absorbance difference that is used. It was demonstrated that the relative error of the Benesi–Hildebrand method in measuring the association constant is often poorly reliable except for the \(K\) values \(<1000^{-1}\) [78, 79]. When the complexation is strong, a nonlinear regression estimation of binding constants is chosen [76].

4.2.5. Fluorescence Spectrometry

Guest fluorescence variation can be the parameter used to calculate the complex stoichiometry [80] and the complex constant [37, 80] by the methods as described earlier.

4.2.6. Affinity Capillary Electrophoresis

Affinity capillary electrophoresis (ACE) can be used to determine the binding constant of an inclusion complex [65, 81]. When a charged solute (guest) is included in a CD cavity, the inclusion complex has a charge identical to that of the free solute but an increased molecular mass. Since the mass-to-charge ratio of the complex is greater than that of the free solute, the mobility of the solute–cyclodextrin (G-CD) complex is lower than that of the free solute. The electrophoretic mobility of a compound G (\(\mu_0\)) is a function of the proportion of the time that this compound is free and the proportion of the time that it is complexed:

\[
\mu_{\text{ep}} = \left(\frac{[G]}{[G] + [G-CD]}\right)\mu_0 + \left(\frac{[G-CD]}{[G] + [G-CD]}\right)\mu_c
\]

where \(\mu_0\) is the electrophoretic mobility of the free guest, \(\mu_c\) the electrophoretic mobility of the G-CD complex, and [G] and [G-CD] the concentrations of the free guest and the inclusion complex, respectively. Given that

\[
[G-CD] = K[G][CD]
\]

then

\[
\mu_{\text{ep}} = \frac{\mu_0 + \mu_c K [CD]}{1 + K [CD]}
\]

where [CD] represents the concentration of CD in the buffer solution. The electrophoretic mobility of the guest, measured from an electropherogram, is

\[
\mu_{\text{app}} = \mu_{\text{ep}} + \mu_{\text{eo}}
\]

and

\[
\mu_{\text{app}} = \frac{LI}{Vt_M}
\]
where \( \mu_{\text{app}} \) is the apparent mobility of the guest, \( L \) and \( l \) the total and effective length of the capillary, respectively, \( V \) the apparent voltage, and \( t_M \) the migration time of the guest. Furthermore, \( \mu_{eo} \) is the mobility of the electrophoretic flow, calculated from the \( t_M \) of a neutral compound (\( t_{eo} \)). Experimentally, \( \mu_{eo} \) is determined using the \( t_M \) of the peak corresponding to water and the equation

\[
\mu_{eo} = \frac{LI}{Vt_{eo}}
\]

The determination of the \( K \) values is achieved by calculation of \( \mu_{eo} \) of the guest in buffers containing increasing concentrations of CD. The data are analyzed by nonlinear regression to assess the agreement with the theoretical model and determine values for \( \mu_0 \), \( \mu_e \), and \( K \) [81].

4.2.7. Isothermal Titration Calorimetry The formation of an inclusion complex is associated with changes in thermodynamic parameters [82]. Isothermal titration calorimetry, a powerful and versatile method for the study of molecular interactions [83], has been used to determine not only the thermodynamic parameters of guest/CD complexation [84], but also to calculate the affinity constant of complexes [85]. During an isothermal titration calorimetry (ITC) experiment, the heat generated or absorbed during a binding reaction is measured. For the experiment, a CD solution (titrant) is added to a guest solution (titrate) over time using one or more individual injections. The heat can be measured either as a change in temperature or as the change in power necessary to maintain the sample and the reference cell at the same temperature. The energy is converted into a binding enthalpy. Calculation of the enthalpy observed includes not only the heat of binding but also any additional heat sources associated with the reaction, including solvent effects, molecular reorganization and conformational changes, heats of dilution, and mechanical artifacts. Thus, careful preparation of solutions and measurement of appropriate background heats are required to obtain thermodynamic parameters that accurately reflect the event(s) of interest [83]. The titration can be either continuous or sequential. The heat produced during each injection is proportional to the amount of complex formed. The change in heat during the experiment allows evaluation of the stoichiometry of interaction, the affinity constant \( K \), and the enthalpy (\( \Delta H \)) of the interaction, from which the entropy (\( \Delta S \)) and the Gibbs free energy of the progress (\( \Delta G \)) can be derived [85].

4.3. Structure of the Complex Most often the guest molecule is not totally included in the CD cavity; the part inside is hindered from any surrounding influence (i.e., humidity, oxidation, pH, etc.) when the part outside can be subjected to all these phenomenon. For this reason it is of prominent interest to know the exact structure of the complex.

4.3.1. NMR and ROESY Studies If classical NMR studies enable evidence for the existence of an inclusion, it does not give direct information on the inclusion structure. ROESY (Rotating frame Overhauser Effect SpectroscopY) experiments provide structural information and allow study of the complex geometry in aqueous solutions. It is a two-dimensional method in which a cross peak can be observed between the protons when the internuclear distance is smaller than about 3 to 4 A [59]. The intensities of the cross peaks depend on the distance between the interacting nuclei, the intensity decreasing with the distance [86]. This method is now a reference for determination of an inclusion complex structure [11, 14, 23, 74, 86].

Cross peaks are displayed between the inner CD protons H3 and H5 and the interacting protons of the guest. For example, in the case of taginin/\( \beta \)-Cd complex [77] it has been shown that the internal H3 proton is correlated with the protons of the lactone part and the unsaturated ketone cycle, while the internal H5 proton is correlated with the protons of the ester part, which could suggest that the taginin is inserted deeply into the cavity by the largest rim, where secondary hydroxyl groups are present, with the ester and lactone parts oriented toward the primary hydroxyl groups of the CD.

4.3.2. Molecular Modeling Molecular modeling makes it possible to obtain the possible geometric structures of the inclusion complex with the docking energies. It constitutes a powerful method to use to predict or explain the inclusion mechanism and the complex structure [52, 65, 71, 77, 87, 88]. Molecular modeling enables geometrical representation of the most probable complex structure (Fig. 7). They are based on the search for a correlation between experimentally determined equilibrium constants of the complexes and some important theoretically evaluated parameters describing the inclusion process, such as the docking energy (gain of potential energy as a consequence of the inclusion), the host–guest contact surfaces (related to the hydrophobic interactions), and the intermolecular interaction fields (related to the hydrophilicity and lipophilicity of the interacting molecules) [87]. It must be emphasized that this method has only a predictive value, which could be useful in preformulation studies to select the best CD to use.

5. CONCLUSIONS CDs are truly exceptional molecules. Not only have they an unusual shape; they are ring molecules, but because of this structure, they have unique properties. They can form inclusion complexes with molecules of size and charge adapted to their cavity. These inclusion complexes are real
molecular encapsulation. Of course, depending on the CD used, the guest compound chosen, and the reaction medium, the inclusion yield can vary and the complex obtained can be a noninclusion complex. The preparation methods are numerous and not necessarily all adapted to the main purpose of the scientist preparing the inclusion. Similarly, the methods used to study the complex are also numerous, but they do not all have the same objective. The general conclusion could be very simple: When preparing and studying an inclusion complex, one must be clear as to his or her objective so as to make the right choice between the various tools proposed in the literature.

REFERENCES


