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CYCLODEXTRINS IN THE PHARMACEUTICAL FIELD

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ABSTRACT

Cyclodextrins (CyDs) are cyclic oligosaccharides, containing a minimum of six D-(+)-glycopyranose units attached by α-1,4-linkages produced by the action of the cyclodextrin-trans-glycosidase enzyme on a medium containing starch. CyDs are somewhat cone-shaped. The outside of CyDs is hydrophilic and the inside of the cavity is hydrophobic in character. If a molecule fits entirely or at least partially into the cavity, an inclusion complex may be formed. In general, hydrophobic molecules, rather than hydrophilic ones, have a higher affinity to the CyD cavity in aqueous solutions. The CyD complexes thus formed are stabilized by various intermolecular forces, such as hydrophobic interaction, van der Waals forces, hydrogen bonding, release of high energy water molecules in complex formation and release of strain energy in the macromolecular CyD ring. Orally administered CyDs have shown to be harmless, because insignificant amounts are absorbed.

Parenterally administered natural CyDs may cause severe nephrotoxicity, particularly β-CyD, due to the formation of low solubility of β-CyD-cholesterol complexes which precipitate in the kidney. Parenterally administered natural CyDs may also cause shape changes and hemolysis of human erythrocytes. Hydroxyalkylated-β-CyDs appear to lack these toxic potentials.

Molecular encapsulation may occur both in the solid state and in solution. Physicochemical properties of the guest molecule may be changed by CyD inclusion complexation. These alterations provide methods to characterize and detect inclusion. There are methods to detect inclusion in solid state and in solution. Some of the methods used to detect inclusion in solution may also be used to determine the complex stability constant. The alteration of physicochemical properties of the guest molecule may be useful to enhance drug properties such as solubility, dissolution rate, bioavailability, stability or to reduce side effects.

1 GENERAL INTRODUCTION

1.1 HISTORY

The first report concerning the isolation of a substance recognizable as cyclodextrin (CyD) was made in 1891 by Villiers [1]. He isolated a small amount of a crystalline substance from a culture medium of Bacillus amylobacter, grown on a medium containing starch. The crystalline substance was named 'cellulosine' because of its resemblance to cellulose. Further progression in the CyD chemistry was made by Schardinger between 1903 and 1911 [2,3]. Schardinger characterized the crystalline substance as a mixture of two cyclic oligosaccharides which he named crystalline dextrin α and crystalline dextrin β. He also published the first detailed description for the preparation and isolation of these cyclic oligosaccharides. CyDs are therefore also known as Schardinger dextrins, cycloamyloses or cycloglucans. A detailed description of the history is given by Clarke et al. [4].

1.2 PRODUCTION

CyDs are formed by the action of the cyclodextrin-trans-glycosidase enzyme (CTG) on a medium containing starch [5,6]. Because of the helical structure of the starch molecules, the primary product of the cleavage by the CTG-enzyme undergoes an intramolecular reaction and α-1,4-linked cyclic products are formed [4].

CyDs are designated by a Greek letter to denote the number of D-glucose units: α- for 6, β-for 7, γ- for 8 and so on for higher CyDs. α-, β- and γ-CyDs are the most commonly used natural CyDs. Higher homologues (nine and more glucose units) do exist but they are difficult to purify and their complexing abilities appear to be poor [4]. CyDs with less than six members cannot be formed for steric reasons [7,8].

The preparation of CyDs can be subdivided in the following main phases [5]:

- cultivation of a micro-organism producing the CTG enzyme;
- separation of the enzyme from the medium, its concentration and purification;
- enzymatic conversion of prehydrolysed starch to a mixture of cyclic and non-cyclic dextrins;
- separation of CyDs from the conversion mixture, their purification and crystallization.

Bacillus macerans is the most frequently used source of the enzyme by which CyDs are now produced, but it is not the only source. Other frequently used producers of the CTG enzyme are Klebsiella pneumoniae and Alkalophilic bacterium No 38-2. The ratios between α-, β- and γ-CyD formed are dependent on the source of the CTG enzyme. Bacillus macerans CTG and Klebsiella pneumoniae CTG produce primary α- and Alkalophilic bacterium No 38-2 CTG primary β-CyD [5]. However, the ratios between the CyDs formed cannot be considered as fixed values. The proportion of the different CyDs formed is also dependent on the incubation time of the enzyme on the starch medium, because most CTG-enzymes initially form α-CyD while the formation of higher CyDs is much slower.

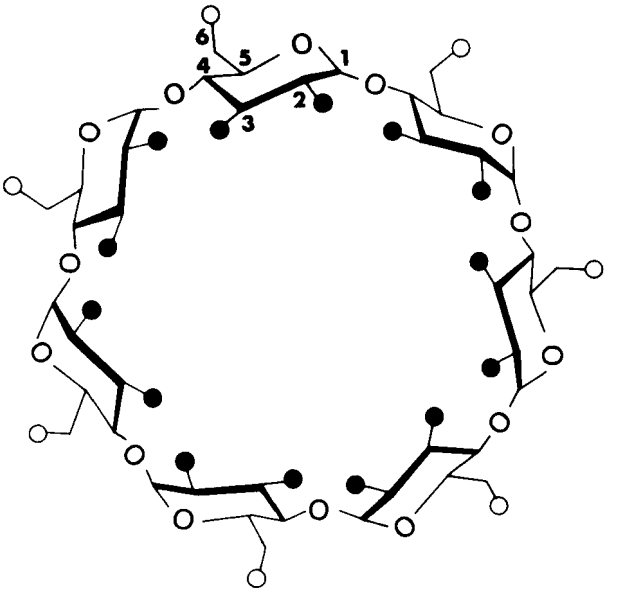

Fig. 1. *The structure and numbering of the atoms of* β-*CyD;* (*o primary and* ●– *secondary hydroxyl groups).*

1.3 STRUCTURE AND PHYSICAL PROPERTIES

As a consequence of the C1-conformation of the α-D-glycopyranosyl residues and the lack of free rotation around glycosidic bonds, the compounds are not perfectly cylindrical molecules, but are somewhat cone-shaped. The secondary hydroxyl groups (on the C-2 and C-3 atoms of the glucose units) are situated on one edge of the ring and all primary hydroxyls on the other. This makes the CyD exterior decidedly hydrophilic [4,9]. The secondary hydroxyl side is wider than the primary hydroxyl side. Free rotation of the primary hydroxyls will reduce the effective diameter of the cavity on the side where they occur, while the secondary hydroxyl groups on relatively rigid chains cannot rotate [5,7,8,10]. The cavity of the torus consists only of a ring of C(3)-H groups, a ring of glucosidic oxygens and another ring of C(5)-H groups. The cavity of the torus is for this reason relatively non-polar (compared to water) [11]. The chemical structure and numbering of the atoms of β-CyD are given in Fig. 1.

TABLE 1. *Physical properties of the CyDs and some derivatives.*

	α	β	γ	DM-β[1)]	HP-β[2)]
* Number of glucose residues:	6	7	8	7	7
* Cavity dimensions (Å)					
- Cavity diameter:	5	6	8	6	6
- Height of torus:	7.9	7.9	7.9	10.0	
- Diameter of periphery:	14.6	15.4	17.5		
* Molecular weight:	973	1135	1297	1331	±1300
* Aqueous solubility [3)]:	14.5	1.85	23.2	57	>50
* Melting point (°C):	275	280	275	295-300	
* pKa [4)]:	12.3	12.2	12.1		
* Half-life of ring opening [5)] (hr):	6.2	5.4	3.0	8.5	
* Enzymatic hydrolysis [6)]:	negli-gible	slow	rapid		

1) - heptakis-2,6-di-O-methyl-β-CyD
2) - 2-hydroxypropyl-β-CyD
3) - in grams per 100 ml water at ambient temperature
4) - pKa: by potentiometry at 25 °C
5) - Half-life of ring opening: in 1 N HCl at 60 °C
6) - by Aspergillus oryzae α-amylase

The dimensions of the CyDs alter with the number of glucose units. Because of their different internal cavity diameters, each CyD shows a different capability of inclusion complex formation with differently sized guest

molecules [12]. Table 1 lists the dimensional sizes as well as some of the important physicochemical properties.

Most striking from Table 1 is the low solubility of β-CyD in water. Many intramolecular hydrogen bonds exist between the secondary hydroxyl groups. The C2-OH group of one glucopyranoside unit can form a hydrogen bond with the C3-OH group of the adjacent glucopyranose unit. These intramolecular H-bonds stabilize the macrocycle of the CyD molecule and turn the CyD molecule into a rigid structure [7]. These H-bonds also prevent hydration of the CyD molecule which is probably an explanation for the low solubility of β-CyD [5,9].

The pKa of the secondary hydroxyls of β-CyD is 12.2 which is low compared to non-cyclic dextrins. This is due to the stabilization of the alkoxide ion by means of the intramolecular hydrogen bonds to neighbouring hydroxyl groups as well as to the combined inductive effects of the electronegative oxygen atoms [13-15]. γ-CyD has a non-coplanar, more flexible structure, and is therefore the most soluble of the three [5]. As a general rule, solubilities of natural CyDs in water increase with increasing temperature [16].

Natural CyDs are completely resistant towards β-amylase since they do not contain end groups susceptible to the attack of this enzyme. However, the enzyme α-amylase is capable of hydrolyzing CyDs, although usually at a very low rate [5,17].

1.4 REQUIREMENTS FOR COMPLEX FORMATION

1.4.1 Geometric compatabilitity

One of the striking features of CyDs is the ability to form inclusion complexes with a variety of compounds, i.e., the trapping of various external molecules (guest molecules) inside the cavity of a CyD (host). The minimum requirement for this inclusion complex formation is that the guest molecule must fit, entirely, or at least partially, into the CyD cavity [18,19]. Stable complexes will not be formed with guest molecules which are too small to be enclosed by the CyD molecules because they will slip out the cavity.

Complex formation is also impossible with molecules which are too bulky to penetrate into the CyD cavity, but if certain groups or side chains of the bulky molecule can penetrate into the CyD cavity, complex formation remains possible [10]. An example of the guest size dependency of inclusion complex formation is the study of Eftink et al. [20]. They determined thermodynamic parameters for the interaction of a congener series of alicyclic carboxylic acids with α- and β- CyD hosts in aqueous solution and found a strong guest-size dependence for both the free energy and enthalpy changes for complex formation. Usually 1:1 (CyD:guest) complexes are formed but when a guest is too long to find complete accommodation in one cavity and its other end is also amenable to complex formation, also 2:1 [21], 2:2 [22], 3:1 [23,24], 3:2 [25], 4:5 [26] etc. complexes can be formed. However, it may also be possible to form 1:2 [27] or three component complexes [28].

1.4.2 Polarity and charge

Not only the stereochemistry but also the polarity of the guest molecules determines whether inclusion may occur. In general, hydrophobic molecules or residues rather than hydrophilic ones have higher affinity to the CyD cavity in aqueous solution [7]. Hydration of a CyD complex with a hydrophobic guest is energetically favoured as compared with the separate hydration of the components. This hydrophobic interaction, is due to the intrinsic cohesion of the water molecules and not to the mutual attraction of the two components.

Cramer et al. [29] described the mechanism of the formation of the inclusion complexes between an azo dye and α-CyD in aqueous solution and divided it into several steps:

1 The approach of the guest to the CyD molecule
2 Breakdown of the water structure inside the CyD ring and removal of some molecules from the ring
3 Breakdown of the water structure around that part of the guest molecule, which is going to be included in the CyD and transport of some water molecules into the solution

4 Interactions of the substituents of the guest molecule with groups on the rim or on the inside of the CyD
5 Possible formation of hydrogen bonds between guest and CyD
6 Reconstruction of the water structure around the exposed parts of the guest after the inclusion process

Usually the complex of an ionic species is much less stable than that of a non-ionized one, the hydrophobic CyD cavity favouring uncharged molecules to enter [31]. Bergeron et al. [30] however, found that sodium p-nitrophenolate binds 13 times more tightly with the CyD cavity than the neutral phenol, while just the opposite is true for benzoic acid and its anion. This contradiction could not only be explained in terms of energy required to move the carboxylate anion from water, a medium with a high dielectric constant to the cycloamylose cavity, a medium of low dielectricity. Further investigations with ^{1}H-NMR by Bergeron et al. [30] showed that the direction of penetration of the guest molecule is responsible for the stability of the p-nitrophenol-CyD complex.

1.5 BINDING FORCE OF THE COMPLEXES

Despite the description of complex formation by hydrophobic interaction, the nature of the binding force remains controversial. Bender and Komiyama [12] state that the interaction force for inclusion complex formation cannot be a classical non-polar binding. Usually non-polar binding is characterized by a very favourable entropy change and inclusion complex formation is associated with a favourable enthalpy change and an unfavourable entropy change [4]. The entropy change consists of several factors. Primary contributions to the change in entropy is that water, originally surrounding the non-polar guest molecule in a highly ordered fashion becomes disordered, and at the same time there are losses in the translational and rotational degrees of freedom because of the association of the host and the guest. These two opposite effects are nearly equal, so the net entropy change may be very small or even zero [5,11]. Thus, inclusion complex formation proceeds by an energetically favoured interaction of a relatively non-polar guest molecule

with an imperfectly solvated hydrophobic cavity. The CyD complexes thus formed should be stabilized by various intermolecular forces such as [5,10-12]:

1 Van der Waals interactions between the guest and host [32,33]. The van der Waals forces here include both permanent induced-dipole-dipole interactions [34] and London dispersion forces [30,35].
2 Hydrogen bonding between the guest and host [32]
3 Release of high energy water molecules in complex formation. In aqueous solution, several water molecules are accommodated in the cavity of CyD. Water molecules in the CyD cavity cannot form so many hydrogen bonds with one another as in the bulk of the solvent, therefore they may be regarded as molecules of enhanced energy or enthalpy. Inclusion complex formation involves the replacement of these high enthalpy water molecules by guest compounds, resulting in a favourable enthalpy change [35,36].
4 Release of strain energy in the macromolecular ring of the CyD (change from the high energy conformation of the CyD-water complex to the lower energy conformation of the CyD-guest complex) [4,10,37]. The macrocyclic conformation of the α-CyD torus in aqueous solution is less symmetrical and should have a higher potential energy. The possible explanation for this higher potential energy content of the water-α-CyD complex is that one of the D-glucopyranose units is rotated into an almost orthogonal position relative to the other five, in order to form hydrogen bonds with the included water molecules and consequently two interglycosidic hydrogen bonds between the O-2 and O-3 atoms are missing.

Saenger et al. [37] reported three possibilities for the formation of an α-CyD inclusion complex. The first possible pathway of α-CyD complex formation in aqueous solution is that the guest molecule directly replaces the two included water molecules. The second possible pathway is that the CyD molecules in aqueous solution absorb energy whereby the CyDs which have formed complexes with water molecules can be assumed as molecules in the relaxed conformation. In such state the water molecules can be easily

substituted by a potential guest molecule. The third possible pathway is that the substrate associates with the outer surface or the edge of the molecule and only penetrates into the cavity after absorbing activation energy.

The possibility of resonance charge delocalization in the guest molecule may also be important in stabilizing a CyD-guest complex. Resonance charge delocalization increases the electron density and polarizability of the substrate molecule which increases the London dispersion forces and the stability of the complex. This was demonstrated by Buvari and Barcza [31] on the dissociated form of p-nitrophenol, that forms a more stable complex with β-CyD than the undissociated form of p-nitrophenol.

Not all forces may contribute equally to the overall binding energy. Bergeron and Rowan [35] showed with ^{1}H-NMR measurements that both London dispersion forces and removal of high energy cavity water contribute substantially to the binding of p-nitrophenolate with α- and β-CyD, while they have not been able to find any support for the strain energy concept [30,35].

Several attempts have been made to determine the extent of the contribution of each phenomenon of the four kinds of interaction in inclusion complex formation but in spite of much effort, there is no general agreement as to the main force stabilizing the inclusion complexes at present [34].

1.6 PREPARATION OF INCLUSION COMPLEXES

There are different methods to prepare inclusion complexes. The most common procedure to prepare inclusion complexes is to stir or shake an aqueous solution of CyD with the guest molecule or its solution. If the system has a B_s type phase solubility diagram [38], the solid inclusion complex can be obtained by precipitation of the microcrystalline powder and this powder is subsequently separated by filtration [19,39-41]. Other methods to prepare solid inclusion complexes are freeze-drying [42-44], spray-drying [45,46] kneading [39,44], coprecipitation [26,42,44,47] and neutralisation [46,48]. A precondition for the freeze-drying and spray drying method is that the complex stoichiometry has to be known. If a suitable quantity of guest

molecules is stirred in a CyD solution of a definite concentration, a real inclusion complex can be obtained. Otherwise, a mixture of guest molecules and the complex or CyD molecules and the complex is obtained. Methods for preparing inclusion complexes without using a solvent are the grinding- [49], melting- [5] and the sealed heating method [26].

1.7 TOXICITY OF NATURAL CYCLODEXTRINS

If CyDs or their inclusion complexes are orally administered, the absorption of the free CyD deserves attention, because the inclusion complexes dissociate under physiological conditions.

Orally administered CyDs have shown to be harmless, probably because insignificant amounts of CyD are absorbed from the intestinal tract [33,50]. Tests to determine the acute toxicity of orally administered natural CyDs on rats resulted in very high LD_{50}-values (>10 g/kg) [7].

A metabolic study conducted with the orally administered ^{14}C-labelled β-CyD and ^{14}C-labelled glucose revealed very limited absorption of the CyD. After administration of labelled glucose, about 5 % of the input radioactivity was detected in the blood within 30 minutes after administration. When labelled β-CyD was administered, less than 0.1 % of the input radioactivity could be detected in the blood within 30 minutes after administration [5,51]. The metabolism of β-CyD occurs by the action of amylases of the bacterial flora of the colon where they are converted to glucose [8], however hydrolysis by α-amylases occurs only at a slow rate [5]. These glucose metabolites may be the source of radioactivity that is measured in the blood of rats after ^{14}C-labelled β-CyD was orally-administered.

Only after parenteral administration of high doses of CyD severe signs of toxicity are observed. This toxicity is characterized by nephrosis. After intravenous administration of 450 mg/kg β-CyD, renal alterations occur in the vacuolar apparatus of the proximal tubuli, accompanied by cytoplasmic vacuolation, cell disintegration and amorphous mineralization. Similar effects have been seen after administration of 1000 mg/kg α-CyD [52]. These histological observations are also made by Frijlink et al. [17]. In electron

microscopic studies Frank et al. [52] found microcrystals in the proximal tubulus after administration of β-CyD. The occurrence of these crystals was directly related to the observed nephrotoxicity, so nephrosis in the kidney should occur as a result of tubular reabsorption of the intact β-CyD which, after concentration in vacuoles, precipitates due to its low aqueous solubility. Intact CyDs should be able to accumulate in the kidney since they are not easily hydrolyzed [8]. Because the occurrence of the crystals was dose dependent, Frank et al. assumed them to consist of pure CyD. However, Frijlink et al. [17] observed also increased amounts of cholesterol in the loop of Henle of β-CyD injected rats. When administered intravenously, CyDs form complexes with cholesterol and with cholesteryl-esters in the bloodstream. These cholesterol-CyD and cholesteryl-CyD complexes can be filtered by the glomular basement membrane and enter the primary urine. In urine, where the concentration of cholesterol is lower than in blood, the complex will dissociate. As a result of the high lipophilicity of cholesterol, it will enter the cells of the proximal tubule or the loop of Henle while most CyD administered is excreted from the body in the urine. A large amount of cholesterol and cholesteryl-esters will be delivered to the kidney through this procedure. Considering this accumulation of cholesterol and the low solubility of the cholesterol-β-CyD complex, the crystals observed by Frank et al. [52] are more likely to consist of complexes formed between the cholesterol or cholesteryl-esters with the small amount of β-CyD remaining in the kidney [17].

Parenterally administered CyDs may also cause shape changes in and hemolysis of human erythrocytes. The hemolytic activity of CyDs was observed to be in the order of β- > α- > γ-CyD in isotonic solutions. Hemolysis was initiated at 3 mM, 6 mM and 16 mM, respectively. To gain insight into the mechanism of CyD-induced hemolysis, the effects of CyDs on membrane components were preliminarily investigated. It was found that CyDs cause the release of some membrane components such as cholesterol, phospholipids and proteins from erythrocytes in the order of β- > γ- > α-CyD. This indicates that CyD induced hemolysis is probably a secondary

event resulting from the membrane disruption which elicited the removal of membrane components from erythrocytes. From the safety point of view, parenteral administration of a large dose of CyDs should be refrained from and γ-CyD seems to be the less harmfull injecting-agent among the three natural CyDs [53].

1.8 CYCLODEXTRIN DERIVATIVES

Natural CyDs can be modified for many different purposes, for example to improve the low aqueous solubility of β-CyD or to decrease the toxicity in parenteral applications. The hydroxyl groups of CyDs are available as starting points of structural modifications and various functional groups have been incorporated into the CyD molecules. For example, alkylated CyDs [8,54-64] and hydroxyalkylated CyDs [65-73] are prepared to obtain better drug carrier properties than natural CyDs. Other examples of CyD derivatives are CyD polymers [33,74] and branched CyDs [75,76]. There are many types of derivatives [10] and only some are described below.

1.8.1 Methylated cyclodextrins

Intramolecular hydrogen bondings are not likely to exist if methyl-groups are introduced onto the hydroxyls of C(2), C(3) or C(6) and the macrocyclic conformation becomes more flexible. Although the permethylation enlarges the whole cavity of the molecule, the methyl groups introduced into the O(3) position extend the O(2), O(3) side of the cavity as well as making the O(6) side narrower [8]. Physicochemical properties are therefore significantly altered. The solubilities of these methylated derivatives are much higher than those of the natural CyDs but they decrease with increasing temperature [8,77,78]. The stabilities of inclusion complexes of di-methylated CyDs are also often higher than for non-modified CyDs [79,80]. The stability constants of complexes of methylated CyDs with guest molecules decrease with increasing temperature [79]. Among a parenteral toxicity study on methylated CyD derivatives, no fundamental differences in the noxious renal effects of β-CyD and its mono- di- and trimethylated derivatives are found, except for

2,6-di-O-methyl-β-CyD (DIMEB) that showed to be less toxic [59]. Methylated CyDs at relatively high concentrations were also found to induce the shape changes of membranes. β-CyD derivatives remove the phospholipids, cholesterol and proteins from the cell surface, depending upon their inclusion abilities (dimethyl-β- > trimethyl-β- > β-CyD). This order is well correlated with their hemolytic activities [8]. No effect on the hemolysis of human erythrocytes was observed up to 1×10^{-2} M concentration, however at 1.7×10^{-2} M, DIMEB resulted in hemolysis. Therefore, on parenteral administration, a local concentration should be avoided and intravenously it should been injected slowly [59]. Being a highly water soluble compound, DIMEB is probably not absorbed. Oral administration of DIMEB to mice did not result in toxic symptoms up to 3000 mg/kg [8,59]. Among methylated CyDs DIMEB has found most applications [54-56,59-62,64].

1.8.2 Hydroxyalkylated cyclodextrins

Other pharmaceutically important CyD derivatives are hydroxyalkylated-β-CyDs (HA-β-CyDs). Examples of these derivatives are 2-hydroxypropyl-β-CyD (HP-β-CyD) and hydroxyethyl-β-CyD (HE-β-CyD). HA-β-CyDs have much higher aqueous solubilities than natural CyDs (>50 %) [68]. HA-β-CyDs are found to be powerful solubilizers of several drugs and no crystalline complexes precipitated at high concentrations of solubilizer, a phenomenon which is often observed when β-CyD is used [68]. A large accumulation of cholesterol and cholesteryl-esters in the kidney occurs when HP-β-CyD is administered parenterally, but the tendency of the HP-β-CyD complex to crystallize is much less than that of the β-CyD complex. This probably explains why the nephrotoxicity of HP-β-CyD is much less than the nephrotoxicity of β-CyD [17]. Also the hemolytic activities of HA-β-CyDs on human erythrocytes are considerably less than that of natural CyDs or DIMEB [68,72]. Consequently, HA-β-CyDs appear to lack the toxic potential of unsubstituted or alkylated CyDs and this means that they may be useful in intravenous (i.v.) and other parenteral preparations [67,70-72,81].

1.8.3 Branched cyclodextrins

Branched β-CyDs, such as glucosyl-β-CyD (G_1-β-CyD) maltosyl-β-CyD (G_2-β-CyD) and di-maltosyl-β-CyD (G_3-β-CyD) have much higher aqueous solubilities than the parent β-CyD. The complexation abilities of G_1-, G_2-, and G_3-β-CyD and their parent CyDs appeared to be almost the same [82]. However, the enhancement of solubility of poorly water-soluble drugs by branched CyDs is much more marked than that by their parent CyDs [75,76]. The reason is that most CyD complexes show typical B_s type solubility curves [38] and precipitates of the complexes appear, whereas the solubilities of drugs in several branched CyD solutions increase linearly as a function of CyD concentration and the solubility curve can be generally classified as being of type A [38]. The hemolytic activity of G_1-β-CyD is about the same as that of β-CyD, but those of the other branched CyDs are lower than that of each parent CyD, and the hemolytic activity becomes weaker with lengthening of the side-chain [75].

1.8.4 Cyclodextrin polymers

Other interesting derivatives are polymeric CyDs. CyD polymers are products containing two or more covalently linked CyD-units. These high molecular weight derivatives of CyDs may be water-soluble and moderately swelling or insoluble and strongly swelling. A water soluble polymer consists of at least two CyD-units and at most about five CyD units. CyDs, having a structure with a three dimensional network, are not soluble in any solvent. These insoluble polymers contain more than five CyD rings in a molecule [5].

CyD polymers are capable to form inclusion complexes with various drug molecules but their complexation behaviour (e.g. complex stability constants) generally differ from those of monomeric CyD [8,84]. CyD polymers can be applied in gel-inclusion and affinity chromatography [33]. CyD polymers, which have excellent disintegrating properties as well as some binding capacity, can also be used as a tabletting aid in direct compression systems as a binder-disintegrant [74,85].

1.9 APPLICATIONS OF CYCLODEXTRIN COMPLEXES

Practical applications of CyDs have gained importance over the past years. The advantages of potential utilizations and the availability of CyDs play a decisive role in the growing interest in CyDs. The practical applications are numerous. CyDs are used in foods, cosmetics and toiletry, pesticides, pharmaceuticals, chemical technology, analytical chemistry, etc. [51,86-88]. The inclusion of a drug results in the modification of its physical and chemical properties. These modifications can be disadvantageous but they are usually not. Some of the advantageous modifications are mentioned below [7,8,33,59,89]:

1 Physicochemical properties of guest molecules can be modified [90]:

- Very volatile substances may be fixated. Consequently, storage and handling will be improved, and the quantities of aromatic and physiological active substances can be better measured out since little or no evaporation takes place.
- Substances which are sparingly soluble in water can be made more soluble and the rate of dissolution may be enhanced by the addition of CyDs. This can lead to a higher pharmaceutical availability and enhanced bioavailability after oral administration.
- The physical stability of suspensions may be increased [91].
- Unpleasant taste and smell may be masked [36].

2 The chemical activities of the guest molecules can be modified:

- Light-, oxygen- or gastric acid sensitive substances may be stabilized.
- The rate of decomposition, hydrolysis, rearrangement etc. may be decreased.
- Incompatible drugs may be mixed together if one of them is protected by CyDs. This also counts for incompatible components of a drug formulation, for example between volatile substances and suppository bases [92].
- Reactions can be made selective by inclusion of functional groups [11,12].

3 Biomedical properties of guest molecules may be modified:

- Side effects [93], local irritation [36], drug-induced hemolysis and muscular tissue damage [94] may be reduced.

4 CyDs can be advantageous in the formulation of drugs:

- A liquid drug may be transformed into a powder form that is suitable for handling and manufacturing.
- Nonhygroscopic powders may be formed which are suitable for tabletting by direct compression [95].
- The content uniformity of a small amount of a drug may be improved by tabletting the microcrystalline complexes.
- Readily soluble products for injectable preparations can be prepared by the freeze-dried CyD complex [96].

The first legislated CyD containing products were the PGE2-β-CyD sublingual tablet (Prostarmon®) and a PGE1-α-CyD injection in Japan [97], but despite the numerous applications of CyDs, there are actually very few pharmaceutical specialities on the market. Duchêne [36] evaluated several factors that might retard the development of the use of CyDs and concluded that except for administrative problems there are no demonstrable reasons for the slow development in using CyDs.

2 DETECTION OF INCLUSION COMPLEX FORMATION AND DETERMINATION OF COMPLEX STABILITY CONSTANTS

2.1 METHODS TO DETECT INCLUSION COMPLEX FORMATION

One of the most interesting properties of CyDs is their ability to form inclusion complexes with a wide variety of guest molecules. Molecular encapsulation may occur both in solution and in the solid state. In solution there is an equilibrium between complexed and non-complexed guest molecules, in solid state guest molecules can be enclosed within the cavity or may be aggregated to the outside of the CyD molecule [29]. Upon inclusion within the CyD cavity, a guest molecule experiences changes in its physicochemical properties. These changes provide methods to detect whether guest molecules are really included in the CyD cavity. Many review articles about methods to characterize or detect inclusion complexation have already

been published [4,5,7,12,19,33,50]. In this chapter, a number of techniques is listed.

2.1.1 Detection of inclusion complexation in the solid state

2.1.1.1 *X-Ray diffractiometry and single crystal X-ray structure analysis*

- Powder X-ray diffractiometry may be used to detect inclusion complexation in the solid state [21,24-26,41,42,46-48,80,98-114]. The method is especially useful in the case of liquid guest molecules since liquids have no diffraction pattern of their own. When the pattern of a newly formed substance clearly differs from that of uncomplexed CyD, complex formation is indicated. When the guest compound is a solid substance, a comparison has to be made between the diffractogram of the assumed complex and that of the mechanical mixture of the guest and CyD molecules. Comparison of the diffractograms is only possible if the CyD as well as guest are, before mixing, treated both under identical conditions as the assumed complex because CyD inclusion complex preparation processes such as freeze drying and grinding, may change the crystallinity of the pure substances and this may also lead to different diffraction patterns [5,17]. A diffraction pattern of a physical mixture is often the sum of those of each component, while the diffraction patterns of CyD complexes are apparently different from each constituent and lead to a 'new' solid phase with a different diffractiogram [21,47,99,103,104,106].

- Single crystal X-ray structure analysis may be used to determine the detailed inclusion structure and mode of interaction [115]. Interactions between the guest and host molecules can be identified and the precise geometrical relationship can be established. Although single crystal X-ray analysis provides much information about inclusion complexes, this technique is too complicated for routine use because the preparation of a single crystal with a suitable size for the analysis is difficult [19,33,116].

2.1.1.2 *Thermo-analytical methods*

Thermo-analytical methods determine whether the guest substance undergoes some change before the thermic degradation of CyD [23-25,42,43,46-48,

98,111,113,114,117-127]. The change of the guest substance may be melting, evaporation, decomposition, oxidation or polymorphic transition [5]. A reversible transformation of β-CyD occurs at 220 °C, thermal decomposition accompanied by oxidation in air starts at 250 °C and melting occurs near 300 °C. Ignition takes place above 300 °C [117].

2.1.1.3 *Thin layer (TLC) and paper chromatography (PC)*

- TLC is used to identify a complex, since complex formation usually diminishes the Rf values of a guest considerably [50]. TLC is not always useful to reveal inclusion complex formation. Inclusion complexation between guest and host molecules is a reversible proces. Consequently, the complex may separate completely in guest and host molecules during the chromatographic process and only the spots of the guest and host molecules are found on the TLC-plate [43].
- PC is also useful to confirm inclusion complexation. This was demonstrated by Mazzi et al. [121] on a complex formed between (RS)-2-(4-isobutyl-phenyl)-propiohydroxamic acid (a non-steroidal antiinflammatory drug, NSAID) and β-CyD.

2.1.1.4 *Infra-red (IR) spectoscopy*

IR spectroscopy is used to assess the interaction between CyD and guest molecules in the solid state [25,26,39,42,43,48,99,105,106,109,113,120, 121,127,129,130,131]. The technique is not generally suitable to detect inclusion complexes and is less clarifying than other methods. CyD bands often change only slightly upon complex formation and if the fraction of guest molecules encapsulated in the complex is less than 25%, bands which could be assigned to the included part of the guest molecules are easily masked by the bands of the spectrum of CyD [33]. The application of IR spectroscopy is limited to guests having some characteristic bands, such as carbonyl or sulfonyl groups [7].

2.1.1.5 *Scanning electron microscopy*

Scanning electron microscopy is used to study the microscopic aspects of the raw materials (CyD and guest substances, respectively) and the product obtained by coprecipitation/evaporation [47,127]. Even if there is a clear difference in crystallization state of the raw material and the product obtained by coprecipitation, this method is inadequate to affirm inclusion complex formation, but nevertheless helps to assess the existence of a single component in the preparations obtained.

2.1.1.6 *Wettability and dissolution tests*

The wetting of the solid phase by a solvent is always the first step of any dissolution process. CyD complexation of a lipophilic drug often improves the wettability in water considerably, but also simple addition of β-CyD to non-wettable solid enhances their wettability. Three methods to characterize the wettability of solid CyD formulations are described by Bajor et al. [132] and Szejtli [5]. These methods include the measurement of the contact angles, powder sedimentation studies which may be carried out by layering equal amounts of the samples onto the surface of water, following their sedimentation photographically and the last method registrates the upward migration of a coloured front of three open tubes containing the guest compound, a mixture of the guest compound with CyD and the inclusion complex, respectively, as function of the time.

When an assumed complex is dispersed in water, a very rapid dissolution is often observed. Dissolution rate tests are based on this observation. The most often used dissolution tests are the rotating disk method [23,48,102,122] and the dispersed amount technique [21,41,44,103,133-135]. In the rotating disk method, the solid CyD formulations are pressed into tablets with exact identical surfaces for the samples and these tablets are placed on a rotating disc apparatus in an aqueous solution. At appropriate intervals samples are removed and analyzed for the guest content. The dispersed amount technique is a similar method but instead of a tablet, a powder is used.

2.1.2 Detection of inclusion complexation in solution

2.1.2.1 *Spectroscopic methods*

- Ultraviolet / visible (UV/VIS) spectroscopy

If complexation causes a change in the absorption spectrum of a guest molecule UV/VIS spectroscopy is a useful method to determine inclusion complexation. Generally the spectral changes observed are similar to the effects caused by changes in the polarity of a solvent, suggesting that the chromophore of the guest is transferred from an aqueous medium to the non-polar CyD cavity [116]. These changes must be due to a perturbation of the electronic energy levels of the guest caused either by direct interaction with the CyD, by the exclusion of solvating water molecules or by a combination of these two effects [4]. Although frequently only small shifts are observed on the UV spectra of included guests [5], the method is often used to detect inclusion complexation [25,29,40,44,58,83,96,106,121,124,125,129-131,136-147].

- Fluorescence spectroscopy

When fluorescent molecules in aqueous solution are included in CyDs, fluorescence spectra may be influenced [28,29,141]. An enhancement in fluorescence is clearly demonstrated by the fluorescence spectra of naphthalene [146], phenprocoumon [147] and ellipticine [137], in absence and in presence of CyDs in aqueous solution.

- Circular dichroism (CD) spectroscopy

CD is a useful method to detect CyD inclusion complexes in aqueous solution [39,40,47,58,96,106,120,124,129-131,144,148,149]. When an achiral guest molecule is included within the asymmetric locus of the CyD cavity which consists of chiral glucose units, new CD bands can be induced in the absorption bands of the optically inactive guest. Not only achiral guest molecules but also chiral guest molecules may show changes in CD spectra upon the formation of inclusion complexes with CyDs [150]. The changes of the (induced) CD of guest molecules following the binding to CyDs may be largely dependent upon the geometry of guest and host molecules [151-154]. Harata and Uedaira [155] investigated the CD spectra of β-CyD complexes

with naphthalene derivatives and found a remarkable difference in the spectra between 1-substituted- and 2-substituted naphthalene complexes, indicating that the steric effects of substituents on the formation of the complex is so strong that the complexation mode may be different for these guest molecules. They suggested that a positive CD band suggests an axial inclusion (parallel to the z-axis of the CyD cavity), while a negative CD band suggests an equatorial inclusion. According to this proposal, 2-substituted naphthalene is estimated to be included axially in the β-CyD cavity. A similar proposal has been made by Kajtar et al. [156], who stated a simple rule for predicting the CD induced in aromatic guests by CyD hosts in inclusion complexes.

- Nuclear magnetic resonance (NMR) spectroscopy

The most direct evidence for the inclusion of a guest into a CyD-cavity in solution is obtained by ^{1}H-NMR spectroscopy [18,46,82,100,109,120,126,129-131,151,157-159]. ^{1}H-NMR may also be used to determine the direction of penetration of guest molecules into the CyD cavity [30,35,106,144,160]. The H-3 and H-5 atoms of CyD, which are directed toward the interior of the CyD-cavity, will show a significant upfield shift if inclusion does indeed occur and the H-1, H-2 and H-4 atoms, located on the exterior of the cavity will show only marginal upfield shifts. Alternatively, if association takes place at the exterior of the torus, H-1, H-2 and H-4 shall be strongly shielded [161].

The spectrum of the guest molecule may also be changed upon inclusion complex formation [162].

A similar method to investigate inclusion complex formation is ^{13}C-NMR spectroscopy [41,157,163-166]. It is often used to gain insight into the inclusion modes of inclusion complexes in aqueous solution [78]. The CyD-induced change in the ^{13}C-chemical shift results predominantly from the electrical environmental effects of the CyD cavity [19] and in general ^{13}C inclusion shifts may be mainly divided into hydrophobic and van der Waals interaction shifts [151].

A ^{1}H-NMR investigation of CyD inclusion complexation may technically be hindered if the complex is slightly soluble in D_2O. ^{13}C-NMR spectra may be obtained in aqueous solutions, which is a great advantage; however, higher concentrations should be required than in ^{1}H-NMR [50].

- Electron spin resonance (ESR)

ESR is a useful method to investigate inclusion complexation with radicals in aqueous solutions [167,168]. The hyperfine coupling constant of radicals is known to be sensitive to the polarity of the medium. If the hyperfine coupling constant alters, the movement of a radical to an environment less polar than water is indicated [169].

2.1.2.2 *pH-potentiometric titration*

If the guest compound has a prototropic function, the potentiometric titration method can be used to detect inclusion complex formation [136,165,170,171]. Due to the fact that CyDs usually favour the unionized guest molecules having a higher hydrophobicity, rather than the ionized ones, the pKa value of an acidic guest molecule is usually increased, while those of basic ones is usually decreased by binding to CyDs.

2.1.2.3 *Electrochemistry*

- Polarography is a suitable method to study inclusion complexation if the electron distribution of a complexed electroactive guest molecule in aqueous solution is different from that in the non-complexed state in aqueous solution and if this change is polarographically detectable [172].

- Conductivity measurements may be used to detect inclusion complexation. Palepu and Richardson [173] found that solution conductivities are dramatically affected by inclusion complex formation with CyDs. They used anionic surfactants having different polar heads, different tail configurations and the same Na^+ counter ion. When these ionic surfactants form inclusion complexes with CyDs, the amphiphilicity of the former often leads to strong associative species that dramatically affect solution conductivities.

2.1.2.4 *Microcalorimetry*

Changes in thermodynamic properties, due to inclusion complexation, can be measured by microcalorimetry. These changes in enthalpy and entropy are associated with the change in behaviour of water following complex formation and include a breakdown of the water structure within the cavity, removal of the water from the cavity, restructuring of water around the guest molecule and release of water into the bulk. Other contributions to the overall energies of reaction are due to the restriction in rotation around the glycosidal linkages of the CyD when the guest molecule enters the cavity. Positive entropies suggest significant contributions from the redistribution of water and large enthalpies should be consistent with dipole interactions and hydrogen bond formation [58,174].

2.1.2.5 *Solubility methods*

In the solubility method, changes in solubility of the guest are plotted as a function of the CyD concentration. If the solubility of a potential guest increases with increasing CyD concentration, complex formation in solution is indicated.

2.1.2.6 *Surface tension technique*

Kaifer et al [139] used a surface tension technique to assess complex formation between surfactants and CyD. The addition of α-CyD caused a quick increase of the surface tension and when the α-CyD concentration was increased to the 4-5 mM level, the surface tension reached values of surfactant free solutions.

2.2 THE COMPLEX STABILITY CONSTANT (K_s)

Effects which can be achieved by means of CyD inclusion complex formation, such as enhancement of the solubility, pharmaceutical availability and bioavailability of a drug, the influence on drug absorption and the stabilization of a drug in solution and in the solid state, all depend on the stability and solubility of the complex. Although complex stability and

solubility are often considered to be close parallel properties, there is no apparent relationship between those two properties [5].

A CyD inclusion complex is always in equilibrium with its free components. The equilibrium state of an 1:1 (host:guest) system is given by the following scheme:

$$CyD + G \rightleftharpoons CyD.G \qquad \text{(scheme 1)}$$

and the general definition of the complex stability, formation or association constant of this equilibrium is given by equation (Eqn 1):

$$K_{s\ (1:1)} = \frac{[CyD.G]}{[CyD]\ [G]} \qquad (1)$$

where CyD, G and CyD.G are the cyclodextrin, guest and inclusion complex, respectively. The complex stability constant is dependent on the temperature and pH. The value of K_s decreases rapidly with increasing temperature due to dissociation of the complex [16,29,79, 143,175,176]. The value of K_s depends also strongly on the pH when the guest is ionizable. If the pH changes, an ionizable group on the guest molecule becomes ionized to a greater extend, giving the guest a charge. This may lead to a partially offset of the desire on the part of the hydrophobic portion of the guest molecules to reside in the CyD cavities [16,58].

Most methods described in 2.1.2 may, provided that the obtained data can be treated quantitatively, be used to determine complex stability constants [177-200]; all occur in solution. An overview of these methods is given by Hirayama and Uekama [19]. If the assumption of the complex stoichiometry is correct, the values obtained by the various methods are more or less comparable. However, if K_s values are obtained by different authors using different methods, the K_s values have been found widely deviating [5].

3 IMPORTANCE OF CYCLODEXTRIN INCLUSION COMPLEXATION FOR PHARMACEUTICAL SCIENCES

Physicochemical properties of guest molecules may be altered if they are surrounded by the hydrophobic environment of a CyD cavity. These alterations may lead to suitable formulations for potential drugs. A drug may dissolve better and faster, have a better bioavailability, fewer side effects and also be more stable. The most obvious possible alteration is an enhancement of the solubility and in pharmacy, this is generally intended to improve bioavailability.

3.1 ENHANCEMENT OF THE SOLUBILITY AND THE RATE OF DISSOLUTION OF POORLY WATER-SOLUBLE DRUGS

The CyD complex of a poorly water-soluble drug is usually more hydrophilic than the free drug itself. It wets more easily and the drug dissolves faster and better [132]. The occurrence of an increased solubility can be concluded from a phase solubility diagram [116] whereas the dissolution rates can be determined by a study of the dissolution kinetics as discussed in section 2.1.1.6.

It is not always necessary to prepare true inclusion complexes to improve dissolution rates. Physical mixtures of slightly water soluble or insoluble drugs and CyDs often have faster dissolution rates than drugs alone. If a crystalline drug is dispersed over a hydrophilic matrix, as is the case with mechanical mix systems with CyDs, this particular drug will be passively carried into the dissolution medium as the carrier dissolves and this may increase the dissolution rate [99,108]. Furthermore, the increase in solubility can still occur through complex formation in solution. The increase in dissolution rate of inclusion complexes may also be due to the decrease in crystallinity of these complexes. A compound in the crystalline state dissolves more slowly than that in the amorphous state [95]. Processes to prepare inclusion complexes, such as grinding and freeze-drying lead to a decrease in crystallinity or even to amorphous powders so in dissolution rate studies

reference compounds such as pure substances or physical mixtures of the pure substances, should be exposed to the same preparation processes as the inclusion complexes. In general, it can be concluded that the increased dissolution rate of CyD-entrapped drug molecules is a result of various factors: an increased solubility, an improved wettability, molecular dispersion and the large surface area available for dissolution [25,113,201]. The higher solubility of the drug may be useful, for example in the preparation of liquid dosage forms such as injections and infusions [96]. There are many examples to demonstrate the effect of CyDs on the solubility and dissolution rate of drugs that are poorly soluble in water. A list of those drugs is given in Table 2.

3.2 ENHANCEMENT OF THE BIOAVAILABILITY

3.2.1 Oral administration

The bioavailability of an orally administered drug depends on several factors, among them the dissolution rate, solubility and the rate of intestinal absorption. Following the oral administration of a drug alone, the drug may dissolve slowly and incompletely in the gastro-intestinal tract. Since orally administered drugs must dissolve in the aqueous medium of the gastro-intestinal tract prior to absorption, the improvement of the solubility and the rate of dissolution of poorly soluble drugs can be seen as first steps towards an improvement in oral bioavailability [202,203].

If the drug itself is very soluble in water, absorption is the rate determining step and CyD complexation does not enhance absorption or may eventually decrease it. On the other hand if the drug is poorly soluble in water, solubility is the rate determining step and the solubility could be increased by CyD complexation to increase the bioavailability [87].

In case of a drug-CyD complex both dissolution and dissociation equilibria determine the amount of free dissolved drug. The degree of dissociation is determined by the complex stability constant, K_s. Dissolving the complex, it partly dissociates untill equilibrium. The inclusion complex with a small complex constant dissociates readily. If the free drug

concentration surpasses thereby its saturation concentration, it will precipitate as a solid substance and inclusion complexation has little or no effect on the overall absorption of the drug. It is also possible that the drug concentration reaches supersaturation during the dissolution process and then decreases gradually due to precipitation of free drug [133]. This kind of dissolution behaviour may provide a variation in drug absorption when the complex is administered orally [84]. It is possible that the extent of absorption, indicated by the area under the plasma concentration time curve (AUC) is the same for the complexed and free drug but that the time needed to reach the peak plasma concentration for the complexed drug is shorter than for the drug alone, due to the faster dissolution of the complex [25]. On the other hand, the larger the stability constant, the lesser the complex dissociates and the lesser the permeation of the drug [204], because the inclusion complex is too bulky and hydrophilic to permeate the hydrophobic membrane (depot-effect). If however the stability constant is large and CyD complex formation is not effective enough in the enhancement of the bioavailability of a certain drug, administration of the CyD complex together with another compound which competes with the drug for the CyD molecule in complex formation may improve the bioavailability of the concerning drug. This is demonstrated by Tokumura et al. [205,206] on the oral bioavailability of the cinnarizine-β-CyD complex with DL-phenylalanine as competiting agent.

Frijlink [17] demonstrated that displacement of the complexed drug from the complex may also occur by lipids in the rectum. The ability of mucus and bile samples to displace was demonstrated in vitro [207] and in vivo results demonstrated that even for drug-CyD complexes with high stability constants significant displacement occurs [17].

In conclusion, rapid dissolution of an inclusion complex may still produce a net increase in drug permeation and cancel out the negative effect due to the poor permeability, particularly in cases where the absorption is dissolution limited [125]. Higher blood levels can be achieved if poorly soluble drugs are solubilized by CyD. If a higher blood level is not desirable, the dose of the drug can be reduced.

3.2.2 Rectal and dermal administration

Reducing the hydrophobicity of drugs by CyD complexation may also improve percutaneous or rectal absorption. A number of reports concerning the enhanced blood or serum levels of a drug after rectal administration of suppositories containing a drug-CyD complex have appeared [78,79,133,208]. The release of a drug from suppositories may be influenced by various factors: drug-vehicle interactions, vehicle composition, solubility, partition coefficient and the particle size in the vehicle. This is demonstrated for flurbiprofen complexes of β-CyD and two methylated β-CyDs in a witepsol H15 suppository base. The release rate of flurbiprofen was significantly improved by inclusion complexation with β-CyD and DIMEB, whilst that wih heptakis (2,3,6-tri-O-methyl)-β-CyD (TRIMEB) was almost the same as for flurbiprofen alone. The different release behaviours between the three complexes might be attributed to the difference in dissolution rate and binding affinity of the complexes to the hydrophobic suppository base [78,79]. The serum levels of the anti-inflammatory drug following rectal administration of hydrophobic witepsol W35 suppositories containing β-CyD or γ-CyD complexes to rabbits have also been higher than those after administration of suppositories containing the drug alone [133]. Uekama et al. [79] investigated the release of flurbiprofen from a hydrophilic polyethylene glycol suppository base. For methylated CyD complexes, the release rates from the polyethylene glycol base were much greater than those from the fatty base, reflecting the lesser interaction between the complex and the hydrophilic base. The relatively high binding affinity of the methylated CyDs to the hydrophobic suppository base may be caused by their high oil solubility compared with natural CyDs. Two other examples concerning the enhancement of the bioavailability after rectal administration are published by Iwaoku et al. [208] and Otagiri et al. [133]. Iwaoku et al. [208] demonstrated that a higher drug concentration in the blood of rats is achieved after administration of witepsol S55 suppositories containing phenobarbital-β-CyD inclusion complex than after administration of suppositories containing phenobarbital alone. Otagiri et al. [133] found that the release of prednisolone

from witepsol H15 suppositories is increased by β- and γ-CyD complexation. Overall it might be concluded that, although CyDs tend to retard the absorption of the drug in solution from the rectum, enhanced absorption of the drug from the complex can be obtained due to fast release from the suppository base due to rapid dissolution [208].

The percutaneous absorption of drugs can be enhanced by CyD complexation. The in vitro release of the dermocorticoid tixocortol 17-butyrate 21-proprionate from two dermal forms is significantly improved after inclusion. After three hours, release is 20% better from an o/w emulsion than from a vaseline base [127]. Similar results are found for betamethasone and beclomethasone diproprionate [110,209]. The in vitro release of betamethasone from gel and hydrophilic ointment bases and of beclomethasone from hydrophilic ointment bases was significant improved by CyD complexation. Permeation and uptake studies indicate that the enhanced release of these drugs may be due to an increase in the apparent rates of dissolution, since the release of the drugs from these bases seems to be diffusion controlled and the lower binding affinity to the ointment bases.

A study based on diffusion revealed that the percutaneous absorption of butylparaben and indomethacin was decreased by β-CyD and DIMEB complexation while that of sulfanilic acid was significantly enhanced by DIMEB complexation [210]. The decrease in penetration of butylparaben was well correlated with the apparent partition coefficient of butylparaben. The free paraben seemed to be capable of penetrating through the skin but its complex does not effectively penetrate into or through the skin. The penetration of sulfanilic acid, which scarcely forms a complex with CyDs was enhanced by DIMEB. This was attributed to the effect of DIMEB on the skin to reduce its barrier function. The same conclusion as for oral and rectal administration can be made: although CyDs tend to retard the penetration through the skin, the fast release of the drug from the ointment base and the rapid dissolution may cancel out the negative effect due to the poor permeability and produce a net increase in drug permeation. CyDs may serve as percutaneous penetration enhancers by varying the skin barrier function.

3.2.3 Parenteral administration

Despite CyDs are extremely useful in many applications, parenteral applications of drugs complexed by natural CyDs are very limited. The solubility of β-CyD is not satisfactory for injectables and it appears to be toxic when given parenterally, precluding its use in intravenous and other parenteral formulations [9]. HP-β-CyD is relatively harmless when administered parenterally and may serve as solubilizer [67,71]. DIMEB has also been reported to be a suitable parenteral drug carrier because it has showned to be less nephrotoxic than β-CyD [59]. The solubilizing effect of CyDs may be useful to prepare liquid dosage forms with higher drug concentrations, which may cause higher blood levels. These higher blood levels may increase the effect; however, if a drug has to pass the blood-brain barrier, CyD complexation may cause a decrease in effect. In vivo studies revealed that the sleeping time of mice (the time until recovery from the loss of the righting reflex) induced with barbituric acid derivatives (BADs) is markedly shortened by simultaneous administration of CyDs. The shortening of the BAD induced sleeping time in presence of CyDs depends upon the magnitude of the stability constants of BAD-CyD complexes obtained in vitro and the sleeping time induced with hexobarbital decreased with an increase in CyDs. Inclusion complex formation of BADs with CyDs should have influence on the distribution of the BAD into the brain and the decrease in sleeping time is said to be due to a lowered BAD distribution to the brain by a decrease of non-complexed BAD [211].

3.3 EFFECTS ON PHYSICAL AND CHEMICAL STABILITY

The active drug content of various formulations may be decreased by degradation processes such as hydrolysis, oxidation, volatilization, sublimation, decomposition by heat, reactions with other components etc. Many drugs can be stabilized against these reactions by CyD complexation, however, CyD complexation of a drug does not always result in stabilization of the drug. The question whether deceleration or acceleration of the

degradation shall occur depends on the inclusion mode of the drug in the CyD cavity. Chin et al. [13] showed that the rate of hydrolysis of ethyl-p-aminobenzoate in aqueous solution was slowed down by CyD encapsulation. Drug molecules forming a true inclusion complex or a complex in which the active center is included in the void of CyD may exhibit a decelerating effect and the hydrolytic rate is primarily dependent on the amount of free ester in solution resulting from dissociation of the complex. On the other hand the ester hydrolysis of acetylsalicylic acid (ASA) in alkaline solution is accelerated by β-CyD. This catalytic effect should be due to the partial fit of ASA in the CyD cavity, leaving the active center sterically fixed in close proximity to a hydroxyl group of CyD. The catalytic effect is related to the degree of ionization of these hydroxyl groups and is absent when the hydroxyl groups are in the unionized form. On this regard, Uekama and Irie [8] stated that methylation of these hydroxyl groups should cause an inhibition rather than acceleration. This hypothesis is supported by the results of Backsenfeld et al. [159], who investigated the effects of various CyDs and CyD derivatives on the stability of indomethacin in phosphate buffer (pH = 7.4). The most favourable ring size to encapsulate indomethacin was that of β-CyD. The β-CyD derivatives inhibit hydrolysis of indomethacin more effectively than the parent CyD. Among the CyD derivatives, those with lipophilic substituents, such as ethyl or methyl, are superior to those with hydrophilic substituents. The more hydroxyl groups of the glucose moiety are substituted, the better the stabilizing effect. However, El-Gendy et al. [54] found that inclusion complexation of ASA in DIMEB resulted in a higher decomposition rate. The decomposition rate of ASA in ground mixtures with DIMEB was also accelerated but this accelerating effect was explained by changes in crystallinity. In the ground mixture and inclusion complex the ASA molecules are dispersed monomolecularly, which results in an increase in the number of reaction sites. Many drugs have already been investigated on their stability upon CyD complexation. A survey is given in Table 2.

TABLE 2. *Effects of CyD complexation on the solubility, dissolution rate and stability of drugs.*

DRUG	CyD	SOL[a]	DISS[b]	STAB[c]	REFERENCE
ANTIBIOTICS/ ANTIMYCOTICS					
amphotericin B	γ	+	+		44,96
anthracyclines	γ			+	192
carbenicillin	α			-	228
	β			-	228
	γ			-	228
chloramphenicol	α	+			229
	β	+			229
cinnamic acid derivatives	α	+	+	+	122,185,189,230
	β	+	+	+/-[1]	122
imidazole derivatives	β	+			231
lankacidin group	β	+			212
mitomycins	γ			+	191
metronidazole	β	+	+	+	91,201
piromidic acid	DIMEB	+	+		126
griseofulvin	α	=			75
	b-α*	=			75,83
	β	+			60,75,83
	b-β*	+			75,83
	γ	+			75
	b-γ*	+			75
	DIMEB	+			60
	TRIMEB	=			60
BARBITURATES	α	+			75,83,211
	b-α	+			75,83
	β	+	+		60,75,83,179,208 211,232-235
	b-β	+			75,83
	DIMEB	+			60
	TRIMEB	+			60
	γ	+			211
BENZODIAZEPINES	α	+			75,83,124,236, 237
	b-α	+			75,83
	β	+		=	9,60,75,76,77 83,124,207, 237,238
	b-β	+			75,76
	γ	+			84,124,237
	b-γ	+			75
	DIMEB	+			9,60,77
	TRIMEB	+			60
	HP-β	+			9

(continued)

Table 2 Continued

DRUG	CyD	SOL[a]	DISS[b]	STAB[c]	REFERENCE
CALCIUM ANTAGONISTS					
diltiazem	DE-β			-	61,135
	TE-β			-	61,135
nifedipine	β	+			60,76
	DIMEB	+			60
	TRIMEB	+			60
	b-β	+			76
nimodipine	β	+	+		67,76
	HE-β	+	+		67
	HP-β	+	+		67
	b-β	+			76
CARDIAC GLYCOSIDES					
digoxin, digitoxin	α	+		+	75,83,109,214
	b-α	+			75,83
	β	+		+	9,75,77,83,109, 213
	b-β	+			75,83
	DIMEB	+		+	9,59,60,77,213
	TRIMEB	+		+	60,213
	HP-β	+		+	69,72,77,213
	γ	+	+	+	75,109,214
	b-γ	+			75
proscillaridin	α	+	+	=	102
	β	+	+	+	102
	DIMEB	+			60
	TRIMEB	+			60
	γ	+	+	+	102
CORTICOSTEROIDS	α	+			75,106,110
	b-α	+			75
	β	+	+	+/=/-[2]	9,45,75,77,106 110,127,193,209 215,235,239,240
	b-β	+			75
	DIMEB	+			9,59,77
	HP-β	+			9,69
	ethyl-β	+			77
	γ	+	+		45,75,106,110, 209,240
	b-γ	+			75
DIURETICS					
thiazide diuretics	β	+	+	=	108,228,238
	DIMEB			-	228
piretanide	β	+			241
	DIMEB	+			241
	HP-β	+			241
furosemide	β	+	+		60,201
	DIMEB	+	+		60,201
	TRIMEB	+			60
	HP-β	+			69
	polymer			+	85

DRUG	CyD	SOL[a]	DISS[b]	STAB[c]	REFERENCE
spironolactone	α	+			23,242
	β	+	+		23,60,201,242
	DIMEB	+			60
	TRIMEB	+			60
	HP-β	+			69
	γ	+	+		23,201,242
FAT SOLUBLE VITAMINS					
Vit D3, cholecalciferol	α	=			75,83
	b-α	=			75,83
	β	+		+	75,83,216, 217,243
	DIMEB	+			243
	b-β	+			75,83
	γ	=			75
	b-γ	+			75
Vit E	α	=			75,83
	b-α	=			75,83
	β	=			75,83
	DIMEB	+			243
	b-β	=			75,83
	γ	=			75
	b-γ	=			75
Vit K1	α	+			75,83,243
	b-α	=			75,83
	β	=			75,83
	DIMEB	+			243
	b-β	=			83
	γ	=			75,243
	b-γ	=			75
Vit K2	β	=			60
	DIMEB	+			60
	TRIMEB	=			60
Vit K3, menadione	α	+			75,83
	b-α	+			75,83
	β	+	+	+	24,39,60,75, 83,217,218
	DIMEB	+			60
	TRIMEB	+			60
	b-β	+			75,83
	γ	+		+	39
	b-γ	+			75
Vit A, retinoic acid	α	+			243
	β	+		=	47,98,243
	DIMEB	+			243
	HP-β	+			69
	γ	=			243

(continued)

Table 2 Continued

DRUG	CyD	SOL[a]	DISS[b]	STAB[c]	REFERENCE
NSAIDs					
AD-1590	α	+			244
	β	+		+	244
	γ	+			244
aspirin	β	+	+		235
	DIMEB	+	-	-	54
diclofenac	β	+	+		60,204
	DIMEB	+			60
	TRIMEB	+			60
flurbiprofen	β	+	+		57,60,78,79,133
	DIMEB	+	+		60,78,79
	TRIMEB	+	+		57,60,78,79
	γ		+		133
ibuprofen	β	+	+		25,204,235,238
indomethacin	β	+	+	+	20,60,77,159,210
	DIMEB	+			60,77,210
	TRIMEB	+			60
	HP-β	+		+	69,77,159
ketoprofen	methyl-β			+	111
naproxen	β	+	+		48,204
RS-2-(4 isobutylphenyl)-propriohydroxamic acid	β	+	+		121
ORAL ANTICOAGULANTS					
warfarin, coumarins	α	+	+	+/-[1]	43,220
	β	+	+	+/-[1]	43,202,220
	HP-β	+			69
	γ			+	220
PROSTAGLANDINS	α	+		+	97,134,163,165, 166,190,245
	β	+	+	+/-[2]	55,56,97,134,163, 165,166,190,245
	DIMEB			+	55,56
	TRIMEB			+	55,56
	γ	+	+	+	41,97,103, 190,166
SEX HORMONES	α	=			75,95
	b-α	=			75
	β	=			60,75,95
	b-β	+			75
	DIMEB	+			59,60
	TRIMEB	+			60
	HP-β		+		69,95
	γ	+			75,95
	b-γ	+			75
MISCELLANEOUS					
buserilin acetate	DE-β	-	-		226
benzocaine	β			+	219

DRUG	CyD	SOL[a]	DISS[b]	STAB[c]	REFERENCE
carmofur	α	+	+	=	21
	β	+	+	+	9,21,80
	DIMEB	+	+	+	9,80
	TRIMEB	=	+		80
	HP-β	+			9
	γ	+	+	=	21
CERM 11884	β	+			100
chlorambucil	HP-β	+		+	66
chlorpromazine	α			-	245,246
	β			-	245,246
	γ			-	245,246
cinnarizine	β	+	+	+	46,247
1,8-dihydroxy-anthraquinone	α	=			141
	β	+			141
	γ	+			141
disulfiram	β	+	+		125
etoposide	α			=	228
	β			=	228
	DIMEB			+	228
	γ			+	228
famotidine	β	+	+		99
guaiazulene	β			+	248
	γ			+	248
iomeglamic acid	β	+	+		249
isosorbide nitrates	β	+	+	+	104,250
	DIMEB	+			9
	HP-β	+			9
	DE-β		-		227
mydeton	β			+	123
phenytoin	β	+	+		60,235,238
	DIMEB	+			60
	TRIMEB	+			60
picotamide	α	+	+		107
	β	+	+		107
	γ	+	+		107
progabide	α	+			221
	β	+		+/-[3]	221
purine nucleosides	α	+/=[2]			144
	β	+			144
	HP-β			+	65
Δ9-tetrahydrocannabinol	β			+	40
tolbutamide	α	+			149
	β	+	+		60,105,113,251
	DIMEB	+			60
	TRIMEB	+			60
ubidecarenone	DIMEB	+	+		62

(continued)

Table 2 Continued

[a] solubility
[b] dissolution rate
[c] stability
* branched CyDs
+ positive effect
- negative effect
= no significant effect

depends on [1] substituent(s) on the guest
[2] the structure of the guest itself
[3] the pH of the degradation medium

3.4 REDUCTION OF THE TOXICITY OF DRUGS

Complexation may decrease toxic side-effects of drug administration. It has been demonstrated that the ulcerogenic character of phenylbutazone [222], salicylic acid [223] and indomethacin [93] could be reduced by β-CyD complexation, local muscle toxicity induced by chlorpromazine [94] could be alleviated by β-CyD complexation and CyDs at relatively low concentrations (<10mM) have protective effects on osmotic and heat induced hemolysis of erythrocytes [53].

CyD complexation may also be used in detoxification processes. Pitha [224,225] described the development of a detoxification process for hypervitaminosis A in mice. Retinoids, compounds related to vitamin A, are effective in treatment of several skin disorders and are possible chemopreventive agents of some forms of cancers. The therapeutic usefulness of retinoids is impeded by their high toxicity. Toxic effects of retinoids are said to occur when these retinoids outnumber their specific carrier proteins, i.e., when there are free retinoids in contact with cells. When mice are treated with high doses of retinoic acid alone, well defined symptoms of hypervitaminosis A are induced and deaths occur within a couple of weeks. If however after about one week DIMEB is applied parenterally, the survival rate of poisoned animals is improved. Obviously, the solubilizer helps to redistribute retinoic acid through the organism, a process which helps to clear the toxin from the poisoned organs and helps to survive.

3.5 SUSTAINED RELEASE

In attempts to design better drug carriers, many CyD derivatives (methylated and hydroxypropylated) and CyD polymers have been prepared. These hydrophilic CyD derivatives are particularly useful to improve the solubility and/or dissolution rate of poorly water-soluble drugs by means of inclusion complex formation. On the other hand, if ethyl groups are introduced onto the hydroxyl groups of β-CyD, its aqueous solubility decreases in proportion to the degree of substitution [61]. These hydrophobic CyD derivatives such as heptakis(2,6-di-O-ethyl)-β-CyD (DE-β-CyD) and heptakis(2,3,6-tri-O-ethyl)-β-CyD (TE-β-CyD), may serve as candidates for sustained release drug carriers. Examples of the possibilities of ethylated CyDs as sustained release drug carriers are given by Uekama et al. [61,135,226,227]. The release rate of diltiazem hydrochloride, a water soluble calcium antagonist, was significantly retarded from compressed tablets by complexation with ethylated-β-CyD. This might be due to the retarded water penetration into the tablets containing the hydrophobic ethylated-β-CyD complexes of diltiazem. In vivo study revealed that the sustained release pattern was produced for a long period without decrease of the AUC [135]. The release of diltiazem could also be controlled by combining the ethylated β-CyD complexes with the parent β-CyD complex in different mixing ratios. Similar results have been obtained for the complex of isosorbide dinitrate and DE-β-CyD complexes [227]. DE-β-CyD may also serve as an injectable sustained release drug carrier [226]. The in vitro release of buselerin acetate, a luteinizing hormone releasing agonist, from an oily suspension was significantly retarded by complexation with DE-β-CyD, mainly due to the poor water solubility of the complex. A single subcutaneous injection of the suspension containing the buselerin-DE-β-CyD complex to rats provided an effective continuous plasma level of buselerin lasting for at least one month, indicating a potentially therapeutic efficacy for the treatment of endocrine-dependent diseases [226].

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