ELSEVIER

Process Biochemistry xxx (2003) xxx–xxx

PROCESS BIOCHEMISTRY

www.elsevier.com/locate/procbio

Review

Cyclodextrins and their uses: a review

E.M. Martin Del Valle

Department of Chemical Engineering, University of Salamanca, Plaza de los Caidos S/N, 37007 Salamanca, Spain

Received 17 February 2003; received in revised form 20 May 2003; accepted 2 July 2003

Abstract

Cyclodextrins are a family of cyclic oligosaccharides composed of α-(1,4) linked glucopyranose subunits. Cyclodextrins are useful molecular chelating agents. They possess a cage-like supramolecular structure, which is the same as the structures formed from cryptands, calixarenes, cyclophanes, spherands and crown ethers. These compounds having supramolecular structures carry out chemical reactions that involve intramolecular interactions where covalent bonds are not formed between interacting molecules, ions or radicals. The majority of all these reactions are of 'host–guest' type. Compared to all the supramolecular hosts mentioned above, cyclodextrins are most important. Because of their inclusion complex forming capability, the properties of the materials with which they complex can be modified significantly. As a result of molecular complexation phenomena CDs are widely used in many industrial products, technologies and analytical methods. The negligible cytotoxic effects of CDs are an important attribute in applications such as rug carrier, food and flavours, cosmetics, packing, textiles, separation processes, environment protection, fermentation and catalysis.

Keywords: Cyclodextrins; Applications; Inclusion complex; Equilibrium; Complexation techniques

1. History

Cyclodextrins are cyclic oligosaccharides consisting of six α-cyclodextrin, seven β-cyclodextrin, eight γ-cyclodextrin or more glucopyranose units linked by α-(1,4) bonds (Fig. 1). They are also known as cycloamyloses, cyclomaltoses and Schardinger dextrins [1,2]. They are produced as a result of intramolecular transglycosylation reaction from degradation of starch by cyclodextrin glucanotransferase (CGTase) enzyme [3].

They were first discovered in 1891 [1], when in addition to reducing dextrins a small amount of crystalline material was obtained from starch digest of *Bacilus amylobacter* "... there is formed in very small amounts (about 3 g/kg of starch) a carbohydrate which forms a beautiful radiate crystals after a few weeks in the alcohol from which the dextrins were precipitated. ... having the composition represented by a multiple of the formula $(C_6H_{10}O_3)\cdot 3H_2O$..." According to other authors, Villiers [1] probably used impure cultures and the cyclodextrins were produced by a *Bacillus macerans* contamination. Villiers [1] named his crystalline product 'cellulosine'. In 1903, Schardinger was able to isolate two crystalline products, dextrins A and B, which were described with regard to their lack of reducing power. The bacterial strain capable of producing these products from starch was unfortunately not maintained.

In 1904, Schardinger [2] isolated a new organism capable of producing acetone and ethyl alcohol from sugar and starch-containing plant material. In 1911, he described that this strain, called *Bacillus macerans*, also produces large amounts of crystalline dextrins (25–30%) from starch. Schardinger [2] named his crystalline products 'crystallised dextrin α' and 'crystallised dextrin β'. It took until 1935 before γ dextrin was isolated. Several fractionation schemes for the production of cyclodextrins [4–6] were also developed. At that time the structures of these compounds were still uncertain, but in 1942 the structures of α and β-cyclodextrin were determined by X-ray crystallography [7]. In 1948, the X-ray structure of γ-cyclodextrin followed and it was recognised that CDs can form inclusion complexes. In 1961, evidence for the natural existence of δ-, ζ-, ξ- and even η-cyclodextrin (9–12 residues) was provided [8]. The main interest in cyclodextrins lies in their ability to form inclusion complexes with several compounds [9–13]. From the X-ray structures it appears that in cyclodextrins the secondary hydroxyl groups (C_2 and C_3) are located on the wider edge of the ring and the primary hydroxyl

E-mail address: emvalle@usal.es (E.M.M. Del Valle).

0032-9592/$ – see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0032-9592(03)00258-9

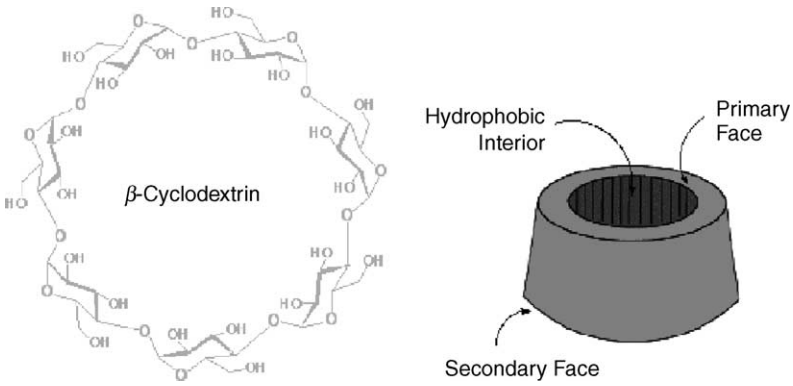

Fig. 1. Chemical structure of β-cyclodextrin.

groups (C_6) on the other edge, and that the apolar C_3 and C_5 hydrogens and ether-like oxygens are at the inside of the torus-like molecules. This result in a molecule with a hydrophilic outside, which can dissolve in water, and an apolar cavity, which provides a hydrophobic matrix, described as a 'micro heterogeneous environment' [14].

As a result of this cavity, cyclodextrins are able to form inclusion complexes with a wide variety of hydrophobic guest molecules. One or two guest molecules can be entrapped by one, two or three cyclodextrins.

2. Properties

Cyclodextrins are of three types: α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin, referred to as first generation or parent cyclodextrins. α-, β- and γ-cyclodextrins are composed of six, seven and eight α-(1,4)-linked glycosyl units, respectively [15]. β-Cyclodextrin is the most accessible, the lowest-priced and generally the most useful. The main properties of those cyclodextrins are given in Table 1.

Studies of cyclodextrins in solution are supported by a large number of crystal structure studies. Cyclodextrins crystallise in two main types of crystal packing, channel structures and cage structures, depending on the type of cyclodextrin and guest compound.

These crystal structures show that cyclodextrins in complexes adopt the expected 'round' structure with all glucopyranose units in the $^4C_1$ chair conformation. Furthermore, studies with linear maltohexaoses, which form an antiparallel double helix, indicate that α-cyclodextrin is the form in which the steric strain due to cyclization is least while γ-cyclodextrin is most strained [3].

Apart from these naturally occurring cyclodextrins, many cyclodextrin derivatives have been synthesised. These derivatives usually are produced by aminations, esterifications or etherifications of primary and secondary hydroxyl groups of the cyclodextrins. Depending on the substituent, the solubility of the cyclodextrin derivatives is usually different from that of their parent cyclodextrins. Virtually all derivatives have a changed hydrophobic cavity volume and also these modifications can improve solubility, stability against light or oxygen and help control the chemical activity of guest molecules [1].

Cyclodextrins are frequently used as building blocks. Up to 20 substituents have been linked to β-cyclodextrin in a regioselective manner. The synthesis of uniform cyclodextrin derivatives requires regioselective reagents, optimisation of reaction conditions and a good separation of products. The most frequently studied reaction is an electrophilic attack at the OH-groups, the formation of ethers and esters by alkyl halides, epoxides, acyl derivatives, isocyanates, and by inorganic acid derivatives as sulphonic acid chloride cleavage of C–OH bonds has also been studied frequently, involving a nucleophilic attack by compounds such as azide ions, halide ions, thiols, thiourea, and amines; this requires activation of the oxygen atom by an electron-withdrawing group [3].

Because of their ability to link covalently or noncovalently specifically to other cyclodextrins, cyclodextrins can be used as building blocks for the construction of supramolecular

Table 1
Cyclodextrins properties

Property	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin
Number of glucopyranose units	6	7	8
Molecular weight (g/mol)	972	1135	1297
Solubility in water at 25 °C (%, w/v)	14.5	1.85	23.2
Outer diameter (Å)	14.6	15.4	17.5
Cavity diameter (Å)	4.7–5.3	6.0–6.5	7.5–8.3
Height of torus (Å)	7.9	7.9	7.9
Cavity volume ($Å^3$)	174	262	427

complexes. Their ability to form inclusion complexes with organic host molecules offers possibilities to build supra molecular threads. In this way molecular architectures such as catenanes, rotaxanes, polyrotaxanes, and tubes, can be constructed. Such building blocks, which cannot be prepared by other methods can be employed, for example, for the separation of complex mixtures of molecules and enantiomers [3].

Each year cyclodextrins are the subject of almost 1000 research articles and scientific abstracts, large numbers of which deal with drugs and drug-related products. In addition, numerous inventions have been described which include cyclodextrins (over 1000 patents or patent applications in the past 5 years). From a regulatory standpoint, a monograph for β-cyclodextrin is already available in both the US Pharmacopoeia/National Formulary (USP 23/NF 18, 1995) the European Pharmacopoeia (3rd ed., 1997). A monograph for 2-hydroxypropyl-*b*-cyclodextrin is in the preparation for US Pharmacopoeia/National Formulary, and various monographs for cyclodextrins are included in compendial sources, e.g. the *Handbook of Pharmaceutical Excipients* [16]. Thus, more than one century after their discovery cyclodextrins are finally, but rapidly, being accepted as 'new' pharmaceutical excipients.

2.1. Toxicological considerations

The safety profiles of the three most common natural cyclodextrins and some of their derivatives have recently been reviewed [17,18]. In general, the natural cyclodextrins and their hydrophilic derivatives are only able to permeate lipophilic biological membranes, such as the eye cornea, with considerable difficulty. Even the somewhat lipophilic randomly methylated β-cyclodextrin does not readily permeate lipophilic membranes, although it interacts more readily with membranes than the hydrophilic cyclodextrin derivatives [19]. All toxicity studies have demonstrated that orally administered cyclodextrins are practically non-toxic, due to lack of absorption from the gastrointestinal tract [17]. Furthermore, a number of safety evaluations have shown that γ-cyclodextrin, 2-hydroxypropyl-*b*-cyclodextrin, sulphobutylether β-cyclodextrin, sulphated β-cyclodextrin and maltosyl β-cyclodextrin appear to be safe even when administered parenterally. However, toxicological studies have also shown that the parent α- and β-cyclodextrin and the methylated β-cyclodextrins are not suitable for parenteral administration.

2.1.1. α-Cyclodextrin

The main properties are: relatively irritating after i.m. injection; binds some lipids; some eye irritation; between 2 and 3% absorption after oral administration to rats; no metabolism in the upper intestinal tract; cleavage only by the intestinal flora of caecum and colon. Excretion after oral administration to rats were: 60% as CO_2 (no CO_2 exhalation after oral administration to germ-free rats), 26–33% as metabolite incorporation and 7–14% as metabolites in faeces and urine, mainly excreted unchanged by the renal route after i.v. injections with $t_{1/2} = 25$ min in rats, LD_{50} oral, rat >10,000 mg/kg, LD_{50} i.v., rat: between 500 and 750 mg/kg.

2.1.2. β-Cyclodextrin

The main properties are: less irritating than α-cyclodextrin after i.m. injection; binds cholesterol; very small amounts (1–2%) absorbed in the upper intestinal tract after oral administration; no metabolism in the upper intestinal tract; metabolised by bacteria in caecum and colon; currently the most common cyclodextrin in pharmaceutical formulations and, thus, probably the best studied cyclodextrin in humans. Application of high doses may be harmful and is not recommended; bacterial degradation and fermentation in the colon may lead to gas production and diarrhoea, LD_{50} oral, rat >5000 mg/kg, LD_{50} i.v., rat: between 450 and 790 mg/kg.

2.1.3. γ-Cyclodextrin

The main properties are: insignificant irritation after i.m. injection; rapidly and completely degraded to glucose in the upper intestinal tract by intestinal enzymes (even at high daily dosages, e.g. 10–20 g/kg); almost no (0.1%) absorption (of intact γ-cyclodextrin) after oral administration; practically no metabolism after i.v. administration; probably the least toxic cyclodextrin, at least of the three natural cyclodextrins. Actively promoted as food additive by its main manufactures; complexing abilities, in general, less than those of β-cyclodextrin and the water soluble β-cyclodextrin derivatives; its complexes frequently have limited solubility in aqueous solutions and tend to aggregate in aqueous solutions, which makes the solution unclear (opalescence) [20], LD_{50} oral, rat $\gg$8000 mg/kg, LD_{50} i.v., rat: about 4000 mg/kg.

2.2. Inclusion complex formation

The most notable feature of cyclodextrins is their ability to form solid inclusion complexes (host–guest complexes) with a very wide range of solid, liquid and gaseous compounds by a molecular complexation [1]. In these complexes (Fig. 2), a guest molecule is held within the cavity of the cyclodextrin host molecule. Complex formation is a dimensional fit between host cavity and guest molecule [21]. The lipophilic cavity of cyclodextrin molecules provides a microenvironment into which appropriately sized non-polar moieties can enter to form inclusion complexes [22]. No covalent bonds are broken or formed during formation of the inclusion complex [23]. The main driving force of complex formation is the release of enthalpy-rich water molecules from the cavity. Water molecules are displaced by more hydrophobic guest molecules present in the solution to attain an apolar–apolar association and decrease of cyclodextrin ring strain resulting in a more stable lower energy state [3].

The binding of guest molecules within the host cyclodextrin is not fixed or permanent but rather is a dynamic

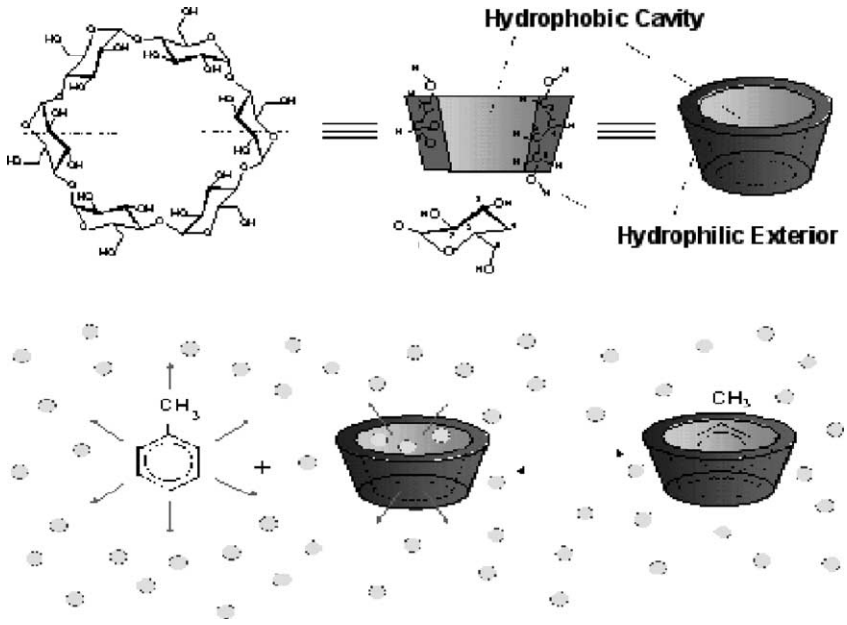

Fig. 2. Cyclodextrins structure and inclusion complex formation.

equilibrium. Binding strength depends on how well the 'host–guest' complex fits together and on specific local interactions between surface atoms. Complexes can be formed either in solution or in the crystalline state and water is typically the solvent of choice. Inclusion complexation can be accomplished in a co-solvent system and in the presence of any non-aqueous solvent. Cyclodextrin architecture confers upon these molecules a wide range of chemical properties markedly different from those exhibited by non-cyclic carbohydrates in the same molecular weight range.

Inclusion in cyclodextrins exerts a profound effect on the physicochemical properties of guest molecules as they are temporarily locked or caged within the host cavity giving rise to beneficial modifications of guest molecules, which are not achievable otherwise [24]. These properties are: solubility enhancement of highly insoluble guests, stabilisation of labile guests against the degradative effects of oxidation, visible or UV light and heat, control of volatility and sublimation, physical isolation of incompatible compounds, chromatographic separations, taste modification by masking off flavours, unpleasant odours and controlled release of drugs and flavours. Therefore, cyclodextrins are used in food [25], pharmaceuticals [26], cosmetics [27], environment protection [28], bioconversion [29], packing and the textile industry [30].

The potential guest list for molecular encapsulation in cyclodextrins is quite varied and includes such compounds as straight or branched chain aliphatics, aldehydes, ketones, alcohols, organic acids, fatty acids, aromatics, gases, and polar compounds such as halogens, oxyacids and amines [24]. Due to the availability of multiple reactive hydroxyl groups, the functionality of cyclodextrins is greatly increased by chemical modification. Through modification, the applications of cyclodextrins are expanded. CDs are modified through substituting various functional compounds on the primary and/or secondary face of the molecule. Modified CDs are useful as enzyme mimics because the substituted functional groups act in molecular recognition. The same property is used for targeted drug delivery and analytical chemistry as modified CDs show increased enantioselectivity over native CDs [1].

The ability of a cyclodextrin to form an inclusion complex with a guest molecule is a function of two key factors. The first is steric and depends on the relative size of the cyclodextrin to the size of the guest molecule or certain key functional groups within the guest. If the guest is the wrong size, it will not fit properly into the cyclodextrin cavity. The second critical factor is the thermodynamic interactions between the different components of the system (cyclodextrin, guest, solvent). For a complex to form, there must be a favourable net energetic driving force that pulls the guest into the cyclodextrin.

While the height of the cyclodextrin cavity is the same for all three types, the number of glucose units determines the internal diameter of the cavity and its volume. Based on these dimensions, α-cyclodextrin can typically complex low molecular weight molecules or compounds with aliphatic side chains, β-cyclodextrin will complex aromatics and heterocycles and γ-cyclodextrin can accommodate larger molecules such as macrocycles and steroids.

In general, therefore, there are four energetically favourable interactions that help shift the equilibrium to form the inclusion complex:

- The displacement of polar water molecules from the apolar cyclodextrin cavity.

- The increased number of hydrogen bonds formed as the displaced water returns to the larger pool.
- A reduction of the repulsive interactions between the hydrophobic guest and the aqueous environment.
- An increase in the hydrophobic interactions as the guest inserts itself into the apolar cyclodextrin cavity.

While this initial equilibrium to form the complex is very rapid (often within minutes), the final equilibrium can take much longer to reach. Once inside the cyclodextrin cavity, the guest molecule makes conformational adjustments to take maximum advantage of the weak van der Waals forces that exist.

Complexes can be formed by a variety of techniques that depend on the properties of the active material, the equilibrium kinetics, the other formulation ingredients and processes and the final dosage form desired. However, each of these processes depends on a small amount of water to help drive the thermodynamics. Among the methods used are simple dry mixing, mixing in solutions and suspensions followed by a suitable separation, the preparation of pastes and several thermo-mechanical techniques.

Dissociation of the inclusion complex is a relatively rapid process usually driven by a large increase in the number of water molecules in the surrounding environment. The resulting concentration gradient shifts the equilibrium in Fig. 2 to the left. In highly dilute and dynamic systems like the body, the guest has difficulty finding another cyclodextrin to reform the complex and is left free in solution.

2.2.1. Equilibrium

The central cavity of the cyclodextrin molecule is lined with skeletal carbons and ethereal oxygens of the glucose residues. It is, therefore, lipophilic. The polarity of the cavity has been estimated to be similar to that of aqueous ethanolic solution [31]. It provides a lipophilic microenvironment into which suitably sized drug molecules may enter and include.

One drug molecule forms a complex with one cyclodextrin molecule.

Measurements of stability or equilibrium constants (K_c) or the dissociation constants (K_d) of the drug–cyclodextrin complexes are important since this is an index of changes in physicochemical properties of a compound upon inclusion. Most methods for determining the *K*-values are based on titrating changes in the physicochemical properties of the guest molecule, i.e. the drug molecule, with the cyclodextrin and then analysing the concentration dependencies. Additive properties that can be titrated in this way to provide information on the *K*-values include [32] aqueous solubility [33–35], chemical reactivity [36,37], molar absorptivity and other optical properties (e.g. optical rotation dispersion), phase solubility measurements [38], nuclear magnetic resonance chemical shifts, pH-metric methods, calorimetric titration, freezing point depression [39], and liquid chromatography chromatographic retention times. While it is possible to use both guest or host changes to generate equilibrium constants, guest properties are usually most easily assessed.

$$\mathrm{D} + \mathrm{CD} \rightleftarrows \mathrm{DCD}$$

$$K_c = \frac{[\mathrm{DCD}]}{[\mathrm{D}][\mathrm{CD}]} \tag{1}$$

Connors has evaluated the population characteristics of cyclodextrin complex stabilities in aqueous solution [40,41].

The stability constant (K_c) is better expressed as $K_{m:n}$ to indicate the stoichiometric ration of the complex. It can be written [32,42]:

$$\underset{(a-mx)}{m\mathrm{L}} + \underset{(b-nx)}{n\mathrm{S}} \overset{K_{m:n}}{\rightleftarrows} \underset{(x)}{\mathrm{L}_m\mathrm{S}_n}$$

So that,

$$K_{m:n} = \frac{[x]}{[a - mx]^m[b - nx]^n} \tag{2}$$

In addition, dissociation constant can also be defined:

$$K_d = \frac{[a - mx]^m[b - nx]^n}{[x]} = \frac{1}{K_c} \quad \text{or} \quad \frac{1}{K_{m:n}} \tag{3}$$

One of the most useful and widely applied analytical approaches in this context is the Phase–solubility method described by Higuchi and Connors [42]. Phase–solubility analysis involves an examination of the effect of a solubilizer, i.e. cyclodextrin or ligand on the drug being solubilized, i.e. the substrate. Experimentally, the drug of interest is added to several vials such that it is always in excess. The presence of solid drug in these systems in necessary to maximise the thermodynamic activity of the dissolved substrate. To the drug or substrate (S) a constant volume of water containing successively larger concentrations of the cyclodextrin or ligand (L) is added. The vials are mixed at constant temperature until equilibrium is established (which frequently takes about 1 week). The solid drug is then removed and the solution assayed for the total concentration of S. A Phase–solubility diagram is constructed by plotting the total molar concentration of S on the *y*-axis and the total molar concentration of L added on the *x*-axis (Fig. 3).

Phase–solubility diagrams prepared in this way fall into two main categories, A- and B-types. A-type curves are indicative for the formation of soluble inclusion complexes while B-type behaviour are suggestive of the formation of inclusion complexes of poor solubility. $\mathrm{AB_S}$-type response denotes complexes of limited solubility and a $\mathrm{B_I}$-curve are indicative of insoluble complexes. The A-curves are subdivided into $\mathrm{A_L}$ (linear increases of drug solubility as a function of cyclodextrin concentration), $\mathrm{A_P}$ (positively deviating isotherm) and $\mathrm{A_N}$ (negatively deviating isotherms) subtypes.

While β-cyclodextrin often gives rise to B-type curves due to the poor water solubility of the ligand itself, the chemically modified CDs including HPβCD and SBEβCD usually produce soluble complexes (i.e. A-type systems). $\mathrm{A_L}$-type

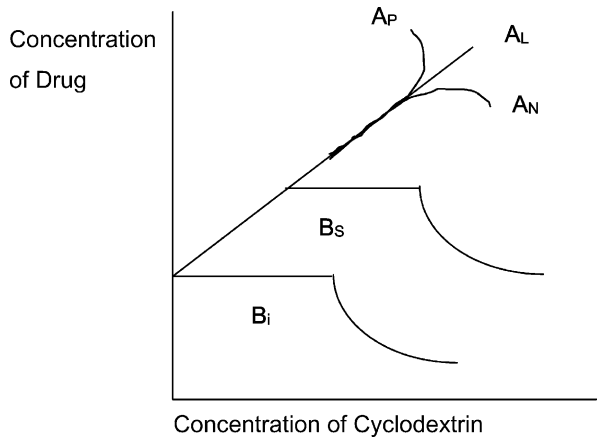

Fig. 3. Phase–solubility relationships.

diagrams are first order with respect to the cyclodextrin (L) and may be first or higher order with respect to the drug (S), i.e. SL, S_2L, S_3L, ..., S_mL. If the slope of an A_L-type system is greater than one, higher order complexes are indicated. A slope of less than one does not necessarily exclude higher order complexation but 1:1 complexation is usually assumed in the absence of other information. A_P-type systems suggest the formation of higher order complexes with respect to the ligand at higher ligand concentrations, i.e. SL_2, SL_3, ..., SL_n. The stoichiometry of A_P-type systems can be evaluated by curve fitting. A_N-type systems are problematic and difficult to interpret.

The negative deviation from linearity may be associated with ligand-induced changes in the dielectric constant of the solvent or self-association of the ligands at high cyclodextrin concentrations.

These Phase–solubility systems not only allows a qualitative assessment of the complexes formed but may also be used to derive equilibrium constants. The equilibrium constant (K) for the formation of $[S_mL_n]$ can be represented by:

$$K = \frac{[S_mL_n]}{[S]^m[L]^n}, \tag{4}$$

where,

$$[S] = S_0 \tag{5}$$

$$[S]_t = S_0 + m[S_mL_n] \tag{6}$$

$$[L]_t = [L] + n[S_mL_n] \tag{7}$$

Therefore, the values of $[S_mL_n]$, [S] and [L] can be obtained:

$$[S] = S_0 \tag{5}$$

$$[S_mL_n] = \frac{[S]_t - S_0}{m} \tag{8}$$

$$[L] = [L]_t - n[S_mL_n], \tag{9}$$

where S_0 is the equilibrium solubility of S (i.e. in the absence of solubilizer), $[S]_t$ is the total concentration of S (complexed and uncomplexed) and $[L]_t$ is the total concentration of L. For Phase–solubility systems that are first order with respect to the cyclodextrin ($n = 1$), the following equation may be derived:

$$[S]_t = \frac{mKS_0^m [L]_t}{1 + KS_0^m} + S_0 \tag{10}$$

A plot of $[S]_t$ versus $[L]_t$ for the formation of S_mL should give a straight line with the y-intercept representing S_0 and the slope being:

$$\text{slope} = \frac{mKS_0^m}{1 + KS_0^m} \tag{11}$$

Therefore, if m is known, K can be calculated. If $m = 1$ (i.e. a 1:1 drug:cyclodextrin complex forms), the following equation can be applied:

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \tag{12}$$

2.2.2. Temperature

The thermodynamic parameters, i.e. the standard free energy change (ΔG), the standard enthalpy change (ΔH) and the standard entropy change (ΔS), can be obtained from the temperature dependence of the stability constant of the cyclodextrin complex [43]. The free energy of reaction is derived from the equilibrium constant using the relationship:

$$\Delta G = -RT \ln K_{1:1} \tag{13}$$

The enthalpies of reactions can likewise be determined from $K_{1:1}$ obtained at various temperatures using the van't Hoff equation. If two sets of data are available (i.e. two K_c values determined at two different temperatures in K) then:

$$\log\left(\frac{K_2}{K_1}\right) = \frac{-\Delta H}{2.303R}\left(\frac{T_2 - T_1}{T_1T_2}\right) \tag{14}$$

On the other hand, if a range of values are available, the ΔH values can be obtained from a plot of $\ln K$ versus $1/T$ using the following relationship:

$$\log K = -\frac{\Delta H}{2.303R}\frac{1}{T} + \text{constant}, \tag{15}$$

where the slope will provide the enthalpy data.

The entropy for the complexation reaction can the be calculated using the expression:

$$\Delta G = \Delta H - T\Delta S \tag{16}$$

Complex formation is usually associated with a relative large negative ΔH and a ΔS, which can either be negative, but also depends on the properties of the guest molecules.

The association of binding constants with substrate polarizability suggest that van der Waal's forces are important in complex formation. Hydrophobic interactions are associated with a slightly positive ΔH and a large positive ΔS and,

therefore, classical hydrophobic interactions are entropy driven suggesting that they are not involved with cyclodextrin complexation since, as indicated, these are enthalpically driven processes. Furthermore, for a series of guests there tends to be a linear relationship between enthalpy and entropy, with increasing enthalpy related with less negative entropy values. This effect, termed compensation, is often correlated with water acting as a driving force in complex formation. However, Connors has pointed out that, in general, the most nonpolar portions of guest molecules are enclosed in the cyclodextrin cavity and, thus, hydrophobic interactions must be important in many cyclodextrin complexes [40]. The main driving force for complex formation is considered by many investigators to be the release of enthalpy-rich water from the cyclodextrin cavity [44]. The water molecules located inside the cavity cannot satisfy their hydrogen bonding potentials and therefore are of higher enthalpy. The energy of the system is lowered when these enthalpy-rich water molecules are replaced by suitable guest molecules which are less polar than water. Other mechanisms that are thought to be involved with complex formation have been identified in the case of α-cyclodextrin.

In this instance, release of ring strain is thought to be involved with the driving force for compound-cyclodextrin interaction. Hydrated α-cyclodextrin is associated with an internal hydrogen bond to an included water molecule which perturbs the cyclic structure of the macrocycle. Elimination of the included water and the associated hydrogen bond is related with a significant release of steric strain decreasing the system enthalpy. In addition, 'non-classical hydrophobic effects' have been invoked to explain complexation [40]. These non-classical hydrophobic effects are a composite force in which the classic hydrophobic effects (characterised by large positive DS) and van der Waal's effects (characterised by negative ΔH and negative ΔS) are operating in the same system. Using adamantanecarboxylates as probes, α-, β- and γ-cyclodextrins were examined. In the case of α-cyclodextrin, experimental data indicated small changes in ΔH and ΔS consistent with little interaction between the bulky probe and the small cavity. In the case of β-cyclodextrin, a deep and snug-fitting complex was formed leading to a large negative ΔH and a near-zero ΔS. Finally, complexation with γ-cyclodextrin demonstrated near-zero ΔH values and large positive ΔS values consistent with a classical hydrophobic interaction. Evidently, the cavity size of γ-cyclodextrin was too large to provide for a significant contribution by van der Waal's-type interactions. These various explanations show that there is no simple construct to describe the driving force for complexation. Although release of enthalpy-rich water molecules from the cyclodextrin cavity is probably an important driving force for the drug-cyclodextrin complex formation other forces may be important. These forces include van der Waals interactions, hydrogen bonding, hydrophobic interactions, release of ring strain in the cyclodextrin molecule and changes in solvent-surface tensions [45].

2.3. Preparation method

Cyclodextrin inclusion is a stoichiometric molecular phenomenon in which usually only one guest molecule interacts with the cavity of the cyclodextrin molecule to become entrapped. A variety of non-covalent forces, such as van der Waals forces, hydrophobic interactions and other forces, are responsible for the formation of a stable complex.

Generally, one guest molecule is included in one cyclodextrin molecule, although in the case of some low molecular weight molecules, more than one guest molecule may fit into the cavity, and in the case of some high molecular weight molecules, more than one cyclodextrin molecule may bind to the guest. In principle, only a portion of the molecule must fit into the cavity to form a complex. As a result, one-to-one molar ratios are not always achieved, especially with high or low molecular weight guests.

2.3.1. Solution dynamics

In the crystalline form, only the surface molecules of the cyclodextrin crystal are available for complexation. In solution, more cyclodextrin molecules become available. Heating increases the solubility of the cyclodextrin as well as that of the guest, and this increases the probability of complex formation. Complexation occurs more rapidly when the guest compound is either in soluble form or in dispersed fine particles.

2.3.2. Temperature effects

Temperature has more than one effect upon cyclodextrin complexes. Heating can increase the solubility of the complex but, at the same time also destabilises the complex. These effects often need to be balanced.

As heat stability of the complex varies from guest to guest, most complexes start to decompose at 50–60 °C, while some complexes are stable at higher temperatures, especially if the guest is strongly bound or the complex is highly insoluble.

2.3.3. Use of solvents

Water is the most commonly used solvent in which complexation reactions are performed. The more soluble the cyclodextrin in the solvent, the more molecules become available for complexation. The guest must be able to displace the solvent from the cyclodextrin cavity if the solvent forms a complex with the cyclodextrin. Water, for example is very easily displaced. The solvent must be easily removed if solvent-free complexes are desired. In the case of multi-component guests, one of the components may act as a solvent and be included as a guest.

Not all guests are readily solubilised in water, making complexation either very slow or impossible. In such cases, the use of an organic solvent to dissolve the guest is desirable. The solvent should not complex well with cyclodextrin and be easily removed by evaporation. Ethanol and diethyl ether are good examples of such solvents.

2.3.4. Effects of water

As the amount of water is increased, the solubility of both cyclodextrin and guest are increased so that complexation occurs more readily. However, as the amount of water is further increased, the cyclodextrin and the guest may be so dilute that they do not get in contact as easily as they do in a more concentrated solution. Therefore, it is desirable to keep the amount of water sufficiently low to ensure complexation occurs at a sufficiently fast rate.

Some high molecular weight compounds such as oils have a tendency to associate with themselves rather than interacting with cyclodextrin. In such cases, using more water allied with good mixing will allow better dispersion and separation of the oil molecules or isolation of the oil molecules from each other. When the oil molecules come into contact with the cyclodextrin, they form a more stable complex than they would if less water were present.

2.3.5. Volatile guests

Volatile guests can be lost during complexation, especially if heat is used. With highly volatile guests, this can be prevented by using a sealed reactor or by refluxing the volatile guests back to the mixing vessel.

2.4. Complexation techniques

Several techniques are used to form cyclodextrin complexes [32,45].

2.4.1. Co-precipitation

This method is the most widely used method in the laboratory. Cyclodextrin is dissolved in water and the guest is added while stirring the cyclodextrin solution. The concentration of β-cyclodextrin can be as high as about 20% if the guest can tolerate higher temperatures. If a sufficiently high concentration is chosen, the solubility of the cyclodextrin–guest complex will be exceeded as the complexation reaction proceeds or as cooling is applied. In many cases, the solution of cyclodextrin and guest must be cooled while stirring before a precipitate is formed.

The precipitate can be collected by decanting, centrifugation or filtration. The precipitate may be washed with a small amount of water or other water-miscible solvent such as ethyl alcohol, methanol or acetone. Solvent washing may be detrimental with some complexes, so this should be tested before scaling up.

The main disadvantage of this method lies in the scale-up. Because of the limited solubility of the cyclodextrin, large volumes of water have to be used. Tank capacity, time and energy for heating and cooling may become important cost factors. Treatment and disposal of the mother liquor obtained after collecting the complex may also be a concern. This can be diminished in many cases by recycling the mother liquor [46,47].

In addition, non-ionic surfactants have been shown to reduce cyclodextrin complexation of diazepam and preservatives to reduce the cyclodextrin complexation of various steroids [48]. On the other hand, additives such as ethanol can promote complex formation in the solid or semisolid state [49]. Un-ionised drugs usually form a more stable cyclodextrin complex than their ionic counterparts and, thus, complexation efficiency of basic drugs can be enhanced by addition of ammonia to the aqueous complexation media. For example, solubilisation of pancratistatin with hydroxypropyl-cyclodextrins was optimised upon addition of ammonium hydroxide [50].

2.4.2. Slurry complexation

It is not necessary to dissolve the cyclodextrin completely to form a complex. Cyclodextrin can be added to water as high as 50–60% solids and stirred. The aqueous phase will be saturated with cyclodextrin in solution. Guest molecules will complex with the cyclodextrin in solution and, as the cyclodextrin complex saturates the water phase, the complex will crystallise or precipitate out of the aqueous phase. The cyclodextrin crystals will dissolve and continue to saturate the aqueous phase to form the complex and precipitate or crystallise out of the aqueous phase, and the complex can be collected in the same manner as with the co-precipitation method.

The amount of time required to complete the complexation is variable, and depends on the guest. Assays must be done to determine the amount of time required. Generally, slurry complexation is performed at ambient temperatures. With many guests, some heat may be applied to increase the rate of complexation, but care must be applied since too much heat can destabilise the complex and the complexation reaction may not be able to take place completely. The main advantage of this method is the reduction of the amount of water needed and the size of the reactor.

2.4.3. Paste complexation

This is a variation of the slurry method. Only a small amount of water is added to form a paste, which is mixed with the cyclodextrin using a mortar and pestle, or on a large scale using a kneader. The amount of time required is dependent on the guest.

The resulting complex can be dried directly or washed with a small amount of water and collected by filtration or centrifugation. Pastes will sometimes dry forming a hard mass instead of a fine powder. This is dependent on the guest and the amount of water used in the paste. Generally, the hard mass can be dried thoroughly and milled to obtain a powdered form of the complex.

2.4.4. Damp mixing and heating

This method uses little or no added water. The amount of water can range from the amount of water of hydration in the cyclodextrin and added guest to up to 20–25% water on a dry basis. This amount of water is typically found in a filter cake from the co-precipitation or slurry methods. The guest and cyclodextrin are thoroughly mixed and placed in

a sealed container. The sealed container and its contents are heated to about 100 °C and then the contents are removed and dried. The amount of water added, the degree of mixing and the heating time have to be optimised for each guest.

2.4.5. Extrusion

Extrusion is a variation of the heating and mixing method and is a continuous system. Cyclodextrin, guest and water can be premixed or mixed as added to the extruder. Degree of mixing, amount of heating and time can be controlled in the barrel of the extruder. Depending upon the amount of water, the extruded complex may dry as it cools or the complex may be placed in an oven to dry.

Extrusion has the advantages of being a continuous process and using very little water. Because of the heat generated, some heat-labile guests decompose using this method.

2.4.6. Dry mixing

Some guests can be complexed by simply adding guest to the cyclodextrin and mixing them together. This works best with oils or liquid guests. The amount of mixing time required is variable and depends on the guest. Generally, this method is performed at ambient temperature and is a variation on the paste method.

The main advantage is that no water need be added, unless a washing step is used. Its disadvantages are the risk of caking on scale-up, resulting in mixing not being sufficiently thorough leading to incomplete complexation, and, with many guests, the length of time required.

2.5. Drying of complexes

The complexes can be dried in an oven, fluid bed dryer or other dryer. Care has to be taken that the complex is not destroyed during the drying process.

2.5.1. Highly volatile guests

For guests with boiling temperatures below 100 °C, a lower temperature must be used during drying. Less guest will be lost during drying when reducing the drying temperature a few degrees below the boiling temperature of the guest.

2.5.2. Spray drying

Complexes can also be spray-dried. Precipitation must be controlled in order to avoid the particles becoming too large and blocking the atomiser or spray nozzle. With volatile guests, some optimisation of drying conditions is required in order to reduce the losses. Spray drying is not a viable means for drying highly volatile and heat-labile guests.

2.5.3. Low temperature drying

A desiccator or freeze dryer may be used to dry complexes. The low temperature minimises the loss of extremely volatile guests. Freeze-drying is especially useful for heat labile guests and soluble complexes such as hydroxypropylated cyclodextrin complexes.

2.6. Release

Once a complex is formed and dried, it is very stable, exhibiting long shelf life at ambient temperatures under dry conditions. Displacement of the complexed guest by another guest requires heating. In many cases, water can replace the guest.

When a complex is placed in water, two steps are involved in the release of the complexed guest. First, the complex is dissolved. The second step is the release of the complexed guest when displaced by water molecules. An equilibrium will be established between free and complexed cyclodextrin, the guest and the dissolved and undissolved complex.

In the case of complexes containing multiple guest components or cyclodextrin types, guest molecules are not necessarily released in the same proportion as in the original guest mixture. Each guest complex may have different solubility and rate of release from the complex. If release rates are different for each component, it is possible to obtain an intended release pattern by alteration of the guest formulation.

2.7. Applications of cyclodextrins

Since each guest molecule is individually surrounded by a cyclodextrin (derivative) the molecule is micro-encapsulated from a microscopical point of view. This can lead to advantageous changes in the chemical and physical properties of the guest molecules.

- Stabilisation of light- or oxygen-sensitive substances.
- Modification of the chemical reactivity of guest molecules.
- Fixation of very volatile substances.
- Improvement of solubility of substances.
- Modification of liquid substances to powders.
- Protection against degradation of substances by micro-organisms.
- Masking of ill smell and taste.
- Masking pigments or the colour of substances.
- Catalytic activity of cyclodextrins with guest molecules.

These characteristics of cyclodextrins or their derivatives make them suitable for applications in analytical chemistry, agriculture, the pharmaceutical field, in food and toilet articles [51].

2.8. Cosmetics, personal care and toiletry

Cosmetic preparation is another area which demands cyclodexytrin use, mainly in volatily suppression of perfumes, room fresheners and detergents by controlled release of fragrances from inclusion compounds.

The major benefits of cyclodextrins in this sector are stabilisation, odour control and process improvement upon

conversion of a liquid ingredient to a solid form. Applications include toothpaste, skin creams, liquid and solid fabric softeners, paper towels, tissues and underarm shields.

The interaction of the guest with CDs produces a higher energy barrier to overcome to volatilise, thus producing long-lasting fragrances [52]. Fragrance is enclosed with the CD and the resulting inclusion compound is complexed with calcium phosphate to stabilise the fragrance in manufacturing bathing preparations [53]. Holland et al. [27] prepared cosmetic compositions containing CDs to create long-lasting fragrances. CD-based compositions are also used in various cosmetic products to reduce body odours [54]. The major benefits of CDs in this sector are stabilisation, odour control, process improvement upon conversion of a liquid ingredient to a solid form, flavour protection and flavour delivery in lipsticks, water solubility and enhanced thermal stability of oils [7]. Some of the other applications include use in toothpaste, skin creams, liquid and solid fabric softeners, paper towels, tissues and underarm shields [3].

The use of CD-complexed fragrances in skin preparations such as talcum powder stabilises the fragrance against the loss by evaporation and oxidation over a long period. The antimicrobial efficacy of the product is also improved [30].

Dry CD powders of size less than 12 mm are used for odour control in diapers, menstrual products, paper towels, etc. and are also used in hair care preparations for the reduction of volatility of odorous mercaptans. The hydoxypropyl β-cyclodextrin surfactant, either alone or in combination with other ingredients, provides improved antimicrobial activity [55].

Dishwashing and laundry detergent compositions with CDs can mask odours in washed items [56,57].

CDs used in silica-based toothpastes increase the availability of triclosan (an antimicrobial) by cyclodextrin complexation and resulting in an almost threefold enhancement of triclosan availability [58]. CDs are used in the preparation of sunscreen lotions in 1:1 proportion (sunscreen/hydroxypropyl β-CD) as the CD's cavity limits the interaction between the UV filter and the skin, reducing the side effects of the formulation. Similarly, by incorporating CD in self-tanning emulsions or creams, the performance and shelf life are improved. An added bonus is that the tan looks more natural than the yellow and reddish tinge produced by traditional dihydroxyacetone products [59].

2.9. Foods and flavours

Cyclodextrins are used in food formulations for flavour protection or flavour delivery. They form inclusion complexes with a variety of molecules including fats, flavours and colours. Most natural and artificial flavours are volatile oils or liquids and complexation with cyclodextrins provides a promising alternative to the conventional encapsulation technologies used for flavour protection. Cyclodextrins are also used as process aids, for example, to remove cholesterol from products such as milk, butter and eggs. Cyclodextrins were reported to have a texture-improving effect on pastry and on meat products. Other applications arise from their ability to reduce bitterness, ill smell and taste and to stabilise flavours when subjected to long-term storage. Emulsions like mayonnaise, margarine or butter creams can be stabilised with α-cyclodextrin. Using β-cyclodextrin may be removed cholesterol from milk; to produce dairy products low in cholesterol [3,30].

Cyclodextrins act as molecular encapsulants, protecting the flavour throughout many rigorous food-processing methods of freezing, thawing and microwaving. β-CD as a molecular encapsulant allows the flavour quality and quantity to be preserved to a greater extent and longer period compared to other encapsulants and provides longevity to the food item [21]. In Japan, cyclodextrins have been approved as 'modified starch' for food applications for more than two decades, serving to mask odours in fresh food and to stabilise fish oils. One or two European countries, for example Hungary, have approved γ-cyclodextrin for use in certain applications because of its low toxicity.

The complexation of CDs with sweetening agents such as aspartame stabilises and improves the taste. It also eliminates the bitter aftertaste of other sweeteners such as stevioside, glycyrrhizin and rubusoside. CD itself is a promising new sweetener. Enhancement of flavour by CDs has been also claimed for alcoholic beverages such as whisky and beer [60]. The bitterness of citrus fruit juices is a major problem in the industry caused by the presence of limonoids (mainly limonin) and flavanoids (mainly naringin). Cross-linked cyclodextrin polymers are useful to remove these bitter components by inclusion complexes.

The most prevalent use of CD in process aids is the removal of cholesterol from animal products such as eggs, dairy products. CD-treated material shows 80% removal of cholesterol. Free fatty acids can also be removed from fats using CDs, thus improving the frying property of fat (e.g. reduced smoke formation, less foaming, less browning and deposition of oil residues on surfaces) [30]. Fruits and vegetable juices are also treated with CD to remove phenolic compounds, which cause enzymatic browning. In juices, polyphenol-oxidase converts the colourless polyphenols to colour compounds and addition of CDs removes polyphenoloxidase from juices by complexation. Sojo et al. [61] studied the effect of cyclodextrins on the oxidation of *o*-diphenol by banana polyphenol oxidase and found that cyclodextrins act as activator as well as inhibitor. By combining 1–4% CD with chopped ginger root, Sung [62] established that it can be stored in a vacuum at cold temperature for 4 weeks or longer without browning or rotting. Flavonoids and terpenoids are good for human health because of their antioxidative and antimicrobial properties but they cannot be utilised as foodstuffs owing to their very low aqueous solubility and bitter taste. Sumiyoshi [63] discussed the improvement of the properties of these plant components (flavanoids and terpenoids) with cyclodextrin complexation. CDs are used in the preparation of foodstuffs

in different ways. For example, highly branched CDs are used in flour-based items like noodles, pie doughs, pizza sheets and rice cakes to impart elasticity and flexibility to dough [25]. They are also used in the preparation of antimicrobial food preservatives containing *trans*-2-hexanalin in apple juice preparation [64] and in the processing of medicinal mushrooms for the preparation of crude drugs and health foods. CDs are used in the preparation of controlled release powdered flavours and confectionery items and are also used in chewing gum to retain flavour for longer duration, a property highly valued by customers [6].

2.10. Pharmaceuticals

A drug substance has to have a certain level of water solubility to be readily delivered to the cellular membrane, but it needs to be hydrophobic enough to cross the membrane.

One of the unique properties of cyclodextrins is their ability to enhance drug delivery through biological membranes. The cyclodextrin molecules are relatively large (molecular weight ranging from almost 1000 to over 1500), with a hydrated outer surface, and under normal conditions, cyclodextrin molecules will only permeate biological membranes with considerable difficulty [65,66]. It is generally recognised that cyclodextrins act as true carriers by keeping the hydrophobic drug molecules in solution and delivering them to the surface of the biological membrane, e.g. skin, mucosa or the eye cornea, where they partition into the membrane. The relatively lipophilic membrane has a low affinity for the hydrophilic cyclodextrin molecules and therefore, they remain in the aqueous membrane exterior, e.g. the aqueous vehicle system (such as o/w cream or hydrogel), salvia or the tear fluid. Conventional penetration enhancers, such as alcohols and fatty acids, disrupt the lipid layers of the biological barrier. Cyclodextrins, on the other hand, act as penetration enhancers by increasing drug availability at the surface of the biological barrier. For example, cyclodextrins have been used successfully in aqueous dermal formulations [67], an aqueous mouthwash solution [68], nasal drug delivery systems [69] and several eye drop solutions [70–72].

The majority of pharmaceutical active agents do not have sufficient solubility in water and traditional formulation systems for insoluble drugs involve a combination of organic solvents, surfactants, and extreme pH conditions, which often cause irritation or other adverse reactions. Cyclodextrins are not irritant and offer distinct advantages such as the stabilisation of active compounds, reduction in volatility of drug molecules, and masking of malodours and bitter tastes.

There are numerous applications for cyclodextrins in the pharmaceuticals field. For example, the addition of α- or β-cyclodextrin increases the water solubility of several poorly water-soluble substances. In some cases this results in improved bioavailability, increasing the pharmacological effect allowing a reduction in the dose of the drug administered.

Inclusion complexes can also facilitate the handling of volatile products. This can lead to a different way of drug administering, e.g. in the form of tablets. Cyclodextrins are used to improve the stability of substances to increase their resistance to hydrolysis, oxidation, heat, light and metal salts. The inclusion of irritating products in cyclodextrins can also protect the gastric mucosa for the oral route, and reduce skin damage for the dermal route.

Furthermore, cyclodextrins can be applied to reduce the effects of bitter or irritant tasting and bad smelling drugs [3,30,73,74].

Administered cyclodextrins are quite resistant to starch degrading enzymes, although they can be degraded at very low rates by α-amylases. α-Cyclodextrin is the slowest, and γ-cyclodextrin is the fastest degradable compound, due to their differences in size and flexibility. Degradation is not performed by saliva or pancreas amylases, but by α-amylases from microorganisms from the colon flora. Adsorption studies revealed that only 2–4% of cyclodextrins were adsorbed in the small intestines, and that the remainder is degraded and taken up as glucose. This can explain the low toxicity found upon oral administration of cyclodextrins [14].

2.11. Agricultural and chemical industries

Cyclodextrins form complexes with a wide variety of agricultural chemicals including herbicides, insecticides, fungicides, repellents, pheromones and growth regulators.

Cyclodextrins can be applied to delay germination of seed. In grain treated with β-cyclodextrins some of the amylases that degrade the starch supplies of the seeds are inhibited. Initially the plant grows more slowly, but later on this is largely compensated by an improved plant growth yielding a 20–45% larger harvest [3]. Recent developments involve the expression of cyclodextrin glucanotransferases (CGTases) in plants [3,30].

In the chemical industry, cyclodextrins are widely used to separate isomers and enantiomers, to catalyse reactions, to aid in various processes and to remove or detoxify waste materials.

Cyclodextrins are widely used in the separation of enantiomers by high performance liquid chromatography (HPLC) or gas chromatography (GC). The stationary phases of these columns contain immobilised cyclodextrins or derived supra molecular architectures. Other analytical applications can be found in spectroscopic analysis. In nuclear magnetic resonance (NMR) studies they can act as chiral shift agents and in Circular Dichroism as selective (chiral) agents altering spectra. In electrochemical chemistry they can be used to mask contaminating compounds, allowing more accurate determinations [3].

One novel use of CDs in catalytic reactions is their ability to serve as enzyme mimics. These are formed by modifying naturally occurring CDs through substituting various functional compounds on the primary or secondary face of the

molecule or by attaching reactive groups. These modified CDs are useful as enzyme mimics because of the molecular recognition phenomenon [3] attributed to the substituted groups on the CD. This ability results from binding of substrates in the hydrophobic cavity with the subsequent reaction initiated by catalytic groups linked to the CD. Rates of reaction are enhanced by almost 1000-fold by such modified CDs versus free solution due to the chelating effect of the CD catalysts. The enantiomeric specificity of CDs in such applications also promises to be a significant attribute [1]. The first chymotrypsin mimic was produced by [75] modifying β-CD, which enhanced the rates of hydrolysis of activated esters and formation of amine bonds by 3.4-folds [76] modified β-CD for the purpose of catalysis and used it for the selective hydroxy-ethylation and hydroxymethylation of phenol. They observed that chemical modification greatly promoted the catalytic activity, and the resulting CD derivative served as a transamine mimic, catalysing the conversion of phenylpyruvic acid to phenylalanine. Atwood [77] explained the use of modified α-cyclodextrin in the reduction of Mn(III) porphyrin.

Due to their steric effects, CDs also play a significant role in biocatalytic processes by increasing the enantioselectivity. After the formation of inclusion complex with the prochiral guest molecule, the preferential attack by the reagent takes place only from one of the enantioselective faces, resulting in higher enantioselectivity. It was reported by Kamal et al. [78] that the hydrolysis of racemic arylpropionic esters by bovine serum albumin, a carrier protein, resulted in low enantioselectivity (50–81% ee), while addition of β-CD to this reaction not only enhanced the enantioselectivity (80–99% ee) but also accelerated the rate of hydrolysis. Rao et al. [79] demonstrated that chiral recognition during cycloaddition reaction of nitriloxides or amines to the C≡C triple bond using baker's yeast as a chiral catalyst was improved by the addition of CDs, increasing the enantioselectivity of yeast by up to 70%.

Cyclodextrins can play a major role in environmental science in terms of solubilisation of organic contaminants, enrichment and removal of organic pollutants and heavy metals from soil, water and atmosphere [80]. CDs are also applied in water treatment to increase the stabilising action, encapsulation and adsorption of contaminants [81]. Using cyclodextrins, highly toxic substances can be removed from industrial effluent by inclusion complex formation. In the mother liquor of the insecticide trichlorfon, the uncrystallisable trichlorfon can be converted into a β-CD complex and in a single treatment 90% of the toxic material is removed [3,30]. Wastewaters containing environmentally unacceptable aromatic compounds such as phenol, *p*-chlorophenol and benzene after treating with β-CD have considerably reduced levels of these aromatic hydrocarbons from their initial levels. Cyclodextrins are used to scrub gaseous effluent from organic chemical industries [3,30]. Solubility enhancement phenomenon of CDs is used for testing of soil remediation. Reid et al. [82] discussed the soil test for determining bioavailability of pollutants using CD and its derivatives. CD complexation also resulted in the increase of water solubility of three benzimidazole-type fungicides (thiabendazole, carbendazim and fuberidazole) making them more available to soil. In addition to its ability to increase the solubility of the hydrocarbon for biodegradation and bioremediation, CDs also decrease the toxicity resulting in an increase in microbial and plant growth. β-Cyclodextrins accelerated the degradation of all types of hydrocarbons influencing the growth kinetics, producing higher biomass yield and better utilisation of hydrocarbon as a carbon and energy source. The low cost, biocompatible and effective degradation makes β-cyclodextrins a useful tool for bioremediation process [83].

2.12. Adhesives, coatings and other polymers

Cyclodextrins increase the tackiness and adhesion of some hot melts and adhesives. They also make additives and blowing agents compatible with hot melt systems. The interaction between polymer molecules in associative thickening emulsion-type coatings such as paints tends to increase viscosity, and CDS can be used to counteract this undesirable effect.

3. Conclusions

Many types of encapsulation are available which coat a fine particle of an active core with an outer shell. Encapsulation also can occur on a molecular level. This can be accomplished using a category of carbohydrates called CDs, encapsulates made with these molecules may possibly hold the key for many future encapsulated formulation solutions.

The ability of cyclodextrins to form inclusion complexes with many guest molecules by taking up a whole molecule, or some part of it, into the cavity place cyclodextrins is a unique class of encapsulation technique. This type of molecular encapsulation will affect many of the physicochemical properties of the guest molecules. The ability of cyclodextrins to form complexes with a wide variety of organic compounds helps to alter the apparent solubility of the molecule, to increase the stability of compound in the presence of light, heat and oxidising conditions and to decrease volatility of compound. These properties have resulted in the growing importance of the applications of cyclodextrins in food, pharmaceutical, agriculture and chromatographic techniques.

The versatility of cyclodextrins and modified cyclodextrins is demonstrated in their range of applications from cosmetics and food to drugs. Recent biotechnological advancements have resulted in dramatic improvements in the efficient manufacture of cyclodextins lowering the cost of these materials making highly purified cyclodextyrins and cyclodextrins derivates available.

In conclusion, due to the unique architecture and the chelating properties, cyclodextrins are becoming an impor-

tant part of the biotechnologist options in the horizons of biocatalysts, encapsulation and control release and in many pharmaceutical applications.

Appendix A. Nomenclature

a, b	molecularity
[CD]	cyclodextrin concentration (mol/l)
[D]	drug concentration (mol/l)
[DCD]	inclusion complex concentration (mol/l)
ΔG	standard free energy change (kcal/kg)
ΔH	standard enthalpy change (kcal/kg)
K_c	stability constant of cyclodextrin complex (l/mol)
K_d	dissociation constant of cyclodextrin complex (mol/l)
$K_{m:n}$	stability constant of stoichiometric cyclodextrin complex (l/mol)
[L]	ligand concentration (mol/l)
$[L]_t$	total ligand concentration (mol/l)
[LS]	complex concentration (mol/l)
m, n	complex order
R	gas constant (cal/mol K)
S_0	equilibrium solubility of S (mol/l)
[S]	substrate concentration (mol/l)
$[S]_t$	total concentration of S (mol/l)
ΔS	standard entropy change (kcal/kg K)
T	temperature (K)

References

[1] Villiers A. Compt Rendu 1891;112:536.
[2] Eastburn SD, Tao BY. Applications of modified cyclodextrins. Biotechnol Adv 1994;12:325–39.
[3] Szetjli J. Introduction and general overview of cyclodextrin chemistry. Chem Rev 1998;98:1743–53.
[4] Stella VJ, Rajewski RA. Cyclodextrins: their future in drug formulation and delivery. Pharm Res 1997;14:556–5567.
[5] Matsuda H, Arima H. Cyclodextrins in transdermal and rectal delivery. Adv Drug Deliv Rev 1999;36:81–99.
[6] Mabuchi N, Ngoa M. Controlled release powdered flavour preparations and confectioneries containing preparations. Japanese Patent JP 128,638 (2001).
[7] Buschmann HJ, Schollmeyer E. Applications of cyclodextrins in cosmetic products: a review. J Cosmet Sci 2002;53:575–92.
[8] Hirose T, Yamamoto Y. Hinokitol containing cyclo-olefin polymer compositions and their molding with excellent antimicrobial and gas barrier properties. Japanese Patent JP 55480 (2001).
[9] Hedges RA. Industrial applications of cyclodextrins. Chem Rev 1998;98:2035–44.
[10] Lu X, Chen Y. Chiral separation of amino acids derivatized with fluoresceine-5-isothiocyanate by capillary electrophoresis and laser-induced fluorescence detection using mixed selectors of beta-cyclodextrin and sodium taurocholate. J Chromatogr A 2002;955:133–40.
[11] Baudin C, Pean C, Perly B, Goselin P. Inclusion of organic pollutants in cyclodextrin and derivatives. Int J Environ Anal Chem 2000;77:233–42.
[12] Kumar R, Dahiya JS, Singh D, Nigam P. Biotransformation of cholesterol using Lactobacillus bulgaricus in glucose controlledbioreactor. Bioresour Technol 2001;28:209–11.
[13] Koukiekolo R, Desseaux V, Moreau Y, Marchis MG, Santimone M. Mechanism of porcine pancreatic alpha-amylase inhibition of amylose and maltopentaose hydrolysis by alpha-, beta- and gamma-cyclodextrins. Eur J Biochem 2001;268:841–8.
[14] Szetjli J. Downstream processing using cyclodextrins. TIBTRCH 1989;7:171–4.
[15] Dass CR, Jessup W, Apolipoprotiens A-I. Cyclodextrins and liposomes as potential drugs for the reversal of atherosclerosis. J Pharm Pharmacol 2000;52:731–61.
[16] Nash RA. Cyclodextrins. In: Wade A, Weller PJ, editors. Handbook of pharmaceutical excipients. London: Pharm. Press & Am. Pharm. Assoc.; 1994. p. 145–8.
[17] Irie T, Uekama K. Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. J Pharm Sci 1997;86:147–62.
[18] Thompson DO. Cyclodextrins-enabling excipients: their present and future use in pharmaceuticals. Crit Rev Ther Drug Carrier Syst 1997;14:1–104.
[19] Totterman AM, Schipper NG, Thompson DO, Mannermaa JP. Intestinal safety of water-soluble β-cyclodextrins in paediatric oral solutions of spironolactone: effects on human intestinal epithelial Caco-2 cells. J Pharm Pharmacol 1997;49:43–8.
[20] Szente L, Szejtli J, Kis GL. Spontaneous opalescence of aqueous γ-cyclodextrin solutions: complex formation or self-aggregation. J Pharm Sci 1998;87:778–81.
[21] Muñoz-Botella S, del Castillo B, Martýn MA. Cyclodetrin properties and applications of inclusion complex formation. Ars Pharm 1995;36:187–98.
[22] Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins: 1. Drug solubilisation and stabilization. J Pharm Sci 1996;85:1017–25.
[23] Schneiderman E, Stalcup AM. Cyclodextrins: a versatile tool in separation science. J Chromatogr B 2000;745:83–102.
[24] Schmid G. Cyclodextrin glucanotransferse production: yield enhancement by overexpression of cloned genes. Trends Biotechnol 1989;7:244–8.
[25] Fujishima N, Kusaka K, Umino T, Urushinata T, Terumi K. Flour based foods containing highly branched cyclodextrins. Japanese Patent JP 136,898 (2001).
[26] Bhardwaj R, Dorr RT, Blanchard J. Approaches to reducing toxicity of parenteral anticancer drug formulations using cyclo-dextrins. J Pharm Sci Technol 2000;54:233–9.
[27] Holland L, Rizzi G, Malton P. Cosmetic compositions comprising cyclic oligosaccharides and fragrance. PCT Int Appl WO 67,716 (1999).
[28] Lezcano M, Ai-Soufi W, Novo M, Rodriguez-Nunez E, Tato JV. Complexation of several benzimidazole-type fungicides with alpha and beta-cyclodextrins. J Agric Food Chem 2002;50:108–12.
[29] Dufosse L, Souchon I, Feron G, Latrasse A, Spinnler HE. In situ detoxification of the fermentation medium during gamma-decalactone production with the yeast Sporidiobolus salmonicolor. Biotechnol Prog 1999;15:135–9.
[30] Hedges RA. Industrial applications of cyclodextrins. Chem Rev 1998;98:2035–44.
[31] Frömming KH, Szejtli J. Cyclodextrins in pharmacy. Topics in inclusion science. Dordrecht: Kluwer Academic Publishers; 1994.
[32] Hirayama F, Uekama K. Methods of investigating and preparing inclusion compounds. In: Duchêne D, editor. Cyclodextrins and their industrial uses. Paris: Editions de Santé; 1987. p. 131–72.
[33] Higuchi T, Connors KA. Phase-solubility techniques. Adv Anal Chem Instrum 1965;4:117–212.
[34] Sigurdardottir AM, Loftsson T. The effect of polyvinylpyrrolidone on cyclodextrin complexation of hydrocortisone and its diffusion through hairless mouse skin. Int J Pharm 1995;126:73–8.

[35] Hussain MA, Diluccio RC, Maurin MB. Complexation of moricizine with nicotinamide and evaluation of the complexation constants by various methods. J Pharm Sci 1993;82:77–9.
[36] Loftsson T. Effects of cyclodextrins on chemical stability of drugs in aqueous solutions. Drug Stabil 1995;1:22–33.
[37] Másson M, Loftsson T, Jónsdóttir S, Fridriksdóttir H, Petersen DS. Stabilisation of drugs through complexation with non-ionic and ionic cyclodextrins. Int J Pharm 1998;164:45–55.
[38] Liu F, Kildsig DO, Mitra AK. Cyclodextrin/weak-electrolyte complexation: interpretation and estimation of association constants from phase solubility diagrams. Pharm Res 1992;9:1671–2.
[39] Suzuki M, Ito K, Fushimi C, Kondo T. The freezing point depression method for the determination of binding parameters of drugs by polyvinylpyrrolidone and polyoxyethylene *n*-dodecylether. Chem Pharm Bull 1993;41:1616–20.
[40] Connors KA. The stability of cyclodextrin complexes in solution. Chem Rev 1997;97:1325–57.
[41] Connors KA. Population characteristics of cyclodextrin complex stabilities in aqueous solution. J Pharm Sci 1995;84:843–8.
[42] Higuchi T, Connors KA. Phase-solubility techniques. Adv Anal Chem Instrum 1965;4:117–212.
[43] Martin A. Physical pharmacy, 4th ed. Philadelphia: Lea & Fedbiger; 1993.
[44] Bergeron RJ. Cycloamylose-substrate binding. Inclision Compounds. London: Academic Press; 1984.
[45] Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J Pharm Sci 1996;85:1017–25.
[46] Loftsson T, Ólafsdóttir BJ, Fridriksdóttir H, Jónsdóttir S. Cyclodextrin complexation of NSAIDS: physicochemical characteristics. Eur J Pharm Sci 1993;1:95–101.
[47] Pitha J, Hoshino T. Effects of ethanol on formation of inclusion complexes of hydroxypropylcyclodextrins with testosterone or with methyl organe. Int J Pharm 1992;80:243–51.
[48] Loftsson T, Stefansdottir Ó, Fridriksdottir H, Gudmundsson Ö. Interaction between preservatives and 2-hydroxypropyl-*b*-cyclodextrin. Drug Devel Ind Pharm 1992;18(13):1477–84.
[49] Furuta T, Yoshii H, Miyamoto A, Yasunishi A, Hirano H. Effects of water of inclusion complexes of *d*-limonene and cyclodextrins. Supramol Chem 1993;1:321–5.
[50] Torres-Labandeira JJ, Davignon P, Pitha J. Oversaturated solutions of drug in hydroxypropylcyclodextrins: parenteral preparation of pancratistatin. J Pharm Sci 1990;80:384–6.
[51] Singh M, Sharma R, Banerjee UC. Biotechnological applications of cyclodextrins. Biotechnol Adv 2002;20:341–59.
[52] Prasad N, Strauss D, Reichart G. Cyclodextrins inclusion for food, cosmetics and pharmaceuticals. European Patent 1,084,625 (1999).
[53] Tatsuya S. Stabilisation of fragrance in bathing preparations. Japanese Patent 11,209,787 (1999).
[54] Trinh J, Dodd TM, Bartolo R, Lucas JM. Cyclodextrin based compositions for reducing body odour. US Patent 5,897,855 (1999).
[55] Woo RAM, Trinh T, Cobb DS, Schneiderman E, Wolff, AM, Rosenbalm EL, Ward TE, Chung AH, Reece S. Uncomplexed cyclodextrin compositions for odour control. US Patent 5,942,217 (1999).
[56] Foley PR, Kaiser CE, Sadler JD, Burckhardt EE, Liu Z. Detergent composition with cyclodextrin perfume complexes to mask malodours. PCT Int Appl WO 01 23,516 (2000).
[57] Angell WF, France, PA. Detergent composition having granular cyclodextrin. PCT Int Appl WO 01 18,163 (2001).
[58] Loftsson T, Leeves N, Bjornsdottir B, Duffy L, Masson M. Effect of cyclodextrins and polymers on triclosan availability and substantivity in toothpastes in vivo. J Pharm Sci 1999;88:1254–8.
[59] Scalia S, Villani S, Casolari A. Inclusion complexation of the sunscreen agent 2-ethylhexyl-*p*-dimethylaminobenzoate with hydroxypropyl-beta-cyclodextrin: effect on photostability. J Pharm Pharmacol 1999;51:1367–74.
[60] Parrish MA. Cyclodextrins—a review. England: Sterling Organics; 1988 (Newcastle-upon-Tyne NE3 3TT).
[61] Sojo MM, Nunez-Delicado E, Garcia-Carmona F, Sanchez-Ferrer A. Cyclodextrins as activator and inhibitor of latent banana pulp polyphenol oxidase. J Agric Food Chem 1999;47:518–23.
[62] Sung H. Composition for ginger preservation. Republic of Korea KR 9,707,148 (1997).
[63] Sumiyoshi H. Utilisation of inclusion complexes with plant components for foods. Nippon Shokuhin Shinsozai Kenkyukaishi 1999;2:109–14.
[64] Takeshita K, Urata T. Antimicrobial food preservatives containing cyclodextrin inclusion complexes. Japanese Patent JP 29,054 (2001).
[65] Frömming KH, Szejtli J. Cyclodextrins in pharmacy. Topics in inclusion science. Dordrecht: Kluwer Academic Publishers; 1994.
[66] Rajewski RA, Stella VJ. Pharmaceutical applications of cyclodextrins. 2. In vivo drug delivery. J Pharm Sci 1996;85:1142–68.
[67] Uekama K, Adachi H, Irie T, Yano T, Saita M. Improved transdermal delivery of prostaglandin E 1 through hairless mouse skin: combined use of carboxymethyl-ethyl-*b*-cyclodextrin and penetration enhancers. J Pharm Pharmacol 1992;44:119–21.
[68] Kristmundsdóttir T, Loftsson T, Holbrook WP. Formulation and clinical evaluation of a hydrocortisone solution for treatment of oral disease. Int J Pharm 1996;139:63–8.
[69] Kublik H, Bock TK, Schreier H, Müller BW. Nasal absorption of 17b-estradiol from different cyclodextrin inclusion formulations in sheep. Eur J Pharm Biopharm 1996;42:320–4.
[70] Loftsson T, Stefánsson E. Effect of cyclodextrins on topical drug delivery to the eye. Drug Devel Ind Pharm 1997;23:473–81.
[71] van Dorne H. Interaction between cyclodextrins and ophthalmic drugs. Eur J Pharm Biopharm 1993;39:133–9.
[72] Jarho P, Urtti A, Pate DW, Suhonen P, Järvinen T. Increase in aqueous solubility. Int J Pharm 1996;137:209–17.
[73] Irie T, Uekama K. Cyclodextrins in peptide and protein delivery. Adv Drug Deliv Rev 1999;36:101–23.
[74] Zhao T, Temsamani J, Agarwal S. Use of cyclodextrin and its derivatives as carriers for oligonucleotide delivery. Antisense Res 1995;5:185–92.
[75] Ekberg B, Anderson L, Mosbach K. The synthesis of an active derivative of cyclomalto hexose for the hydrolysis of esters and the formation of amide bonds. Carbohydr Res 1989;192:111–7.
[76] Morozumi T, Uetsuka H, Komiyama M, Pitha J. Selective synthesis using cyclodextrins as catalyst. 6. Cyclodextrin modification for para-selective hydroxymethylation and hydroxyetheylation of phenol. J Mol Catal 1991;70:399–406.
[77] Atwood JL. Inclusion phenomenon and molecular recognition. New York: Plenum; 1990.
[78] Kamal A, Rabalingum T, Venugopalan N. Enantioselective hydrolysis of aryloxypopionic esters by bovine serum albumin: enhancement in selectivity by β-CD. Tetrahedron: Asymmetry 1991;2:39.
[79] Rao KR, Bhanumathi N, Srinivasan TN, Sattur PB. A regioselective enzyme catalysed cycloaddition. Tetrahedron Letters 1990;31:892–9.
[80] Gao S, Wang L. Application of cyclodextrin in environmental science. Huanjing Kexue Jinzhan 1998;6:80–6.
[81] Wu C, Fan J. Applications of cyclodextrin to water treatment. Shuichuli Jishu 1998;24:67–70.
[82] Reid BJ, Semple KT, Jhones KC. Soil test for determining bioavailability of pollutants. PCT Int Appl WO 99 54,727 (1999).
[83] Bardi L, Mattei A, Steffan S, Marzona M. Hydrocarbon degradation by a soil microbial population with beta-cyclodextrin as surfactant to enhance bioavailability. Enzyme Microb Technol 2000;27:709–13.