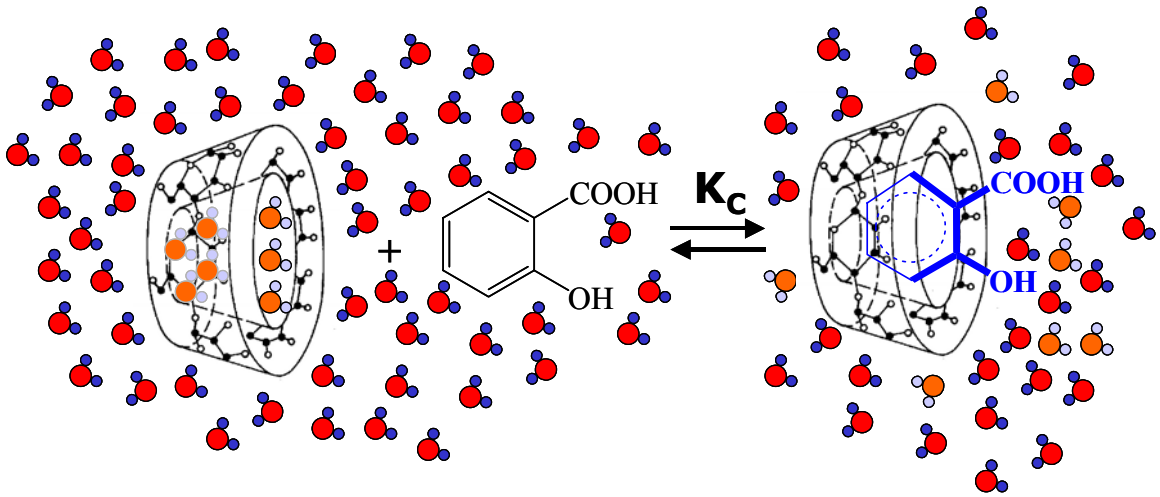
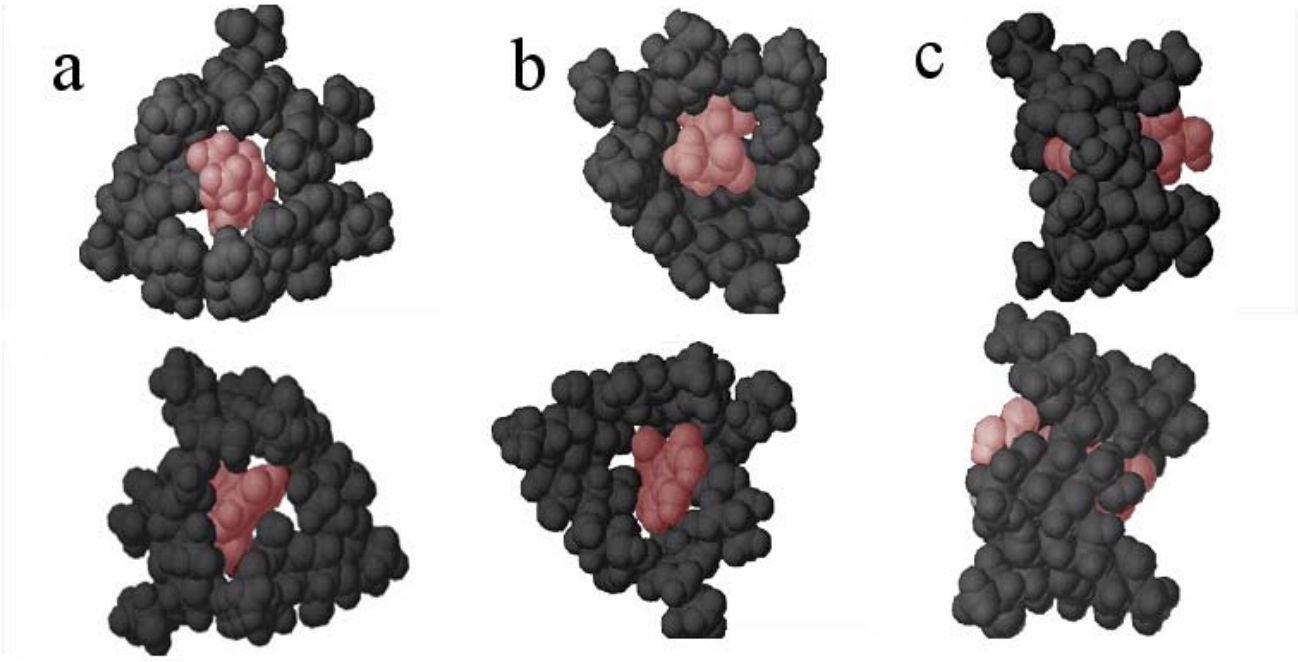
CYCLODEXTRINS



The conventional model of drug/cyclodextrin complex formation (salicylic acid/β-cyclodextrin inclusion complex).



Molecular modeling of diflunisal (below) and ibuprofen (above) in 2-hydroxypropyl-β-cyclodextrin cavity in the gas phase seen from the narrow end (a), the wider end (b) and from the side (c) of the cyclodextrin molecule.

A. Magnúsdóttir, M. Másson and T. Loftsson, *J. Incl. Phenom. Macroc. Chem.* **44**, 213-218, 2002.

Structure

Cyclodextrins are a group of structurally related natural products formed during bacterial digestion of cellulose. These cyclic oligosaccharides consist of (α-1,4)-linked α-D-glucopyranose units and contain a somewhat lipophilic central cavity and a hydrophilic outer surface. Due to the chair conformation of the glucopyranose units, the cyclodextrins are shaped like a truncated cone rather than perfect cylinders. The hydroxyl functions are orientated to the cone exterior with the primary hydroxyl groups of the sugar residues at the narrow edge of the cone and the secondary hydroxyl groups at the wider edge. The central cavity is lined by the skeletal carbons and ethereal oxygens of the glucose residues, which gives it a lipophilic character. The polarity of the cavity has been estimated to be similar to that of an aqueous ethanolic solution. The natural α-, β- and γ-cyclodextrin consist of six, seven, and eight glucopyranose units, respectively. The natural cyclodextrins, in particular β-cyclodextrin, are of limited aqueous solubility meaning that complexes resulting from interaction of lipophiles with these cyclodextrin can be of limited solubility resulting in precipitation of solid cyclodextrin complexes from water and other aqueous systems. In fact, the aqueous solubility of the natural cyclodextrins is much lower than that of comparable acyclic saccharides. This is thought to be due to relatively strong intermolecular hydrogen bonding in the crystal state. Substitution of any of the hydrogen bond forming hydroxyl groups, even by lipophilic methoxy functions, results in dramatic improvement in their aqueous solubility. Cyclodextrin derivatives of pharmaceutical interest include the hydroxypropyl derivatives of β- and γ-cyclodextrin, the randomly methylated β-cyclodextrin, sulfobutylether β-cyclodextrin, and the so-called branched cyclodextrins such as glucosyl-β-cyclodextrin.

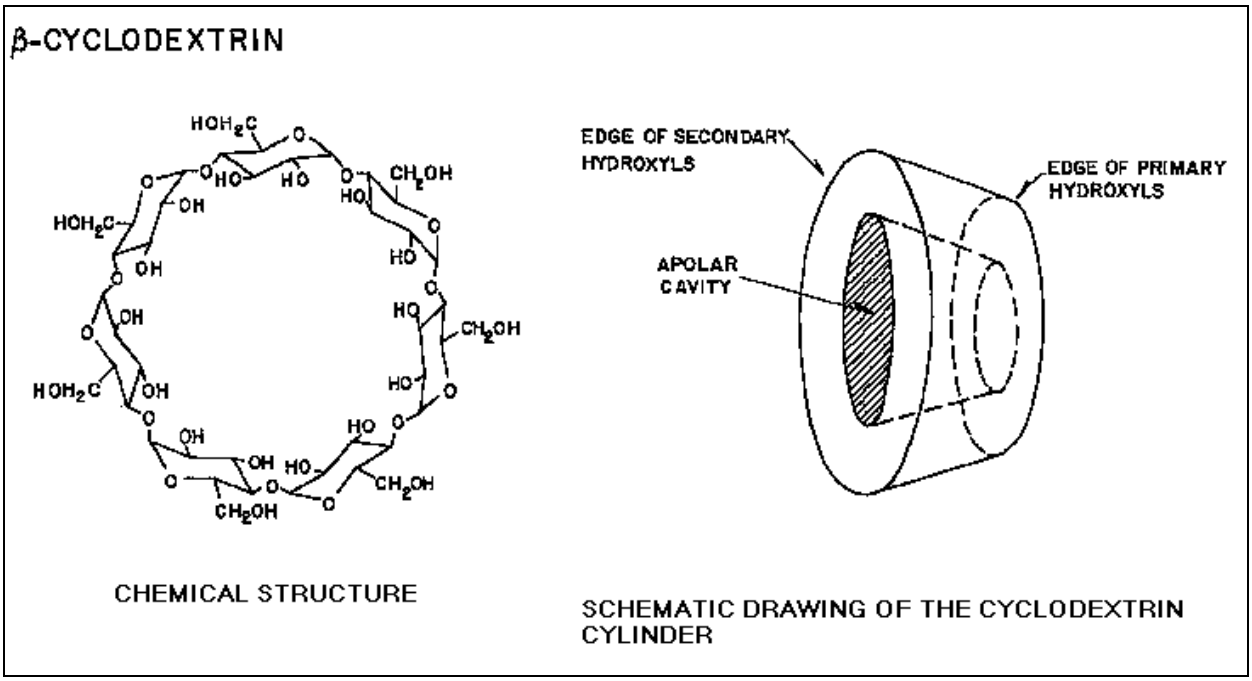
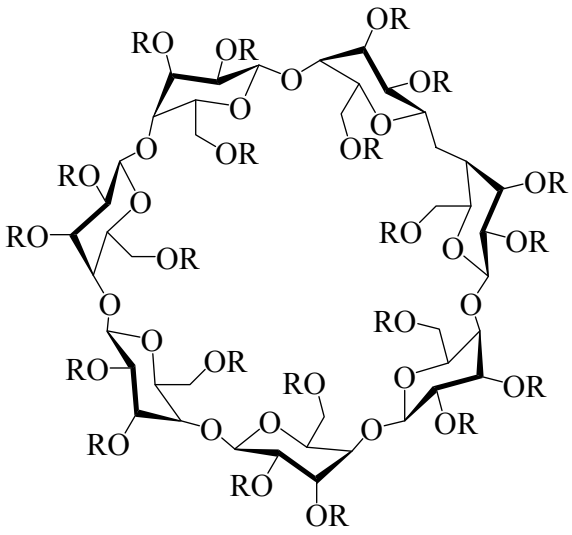


Figure 1. The chemical structure and the molecular shape of β-cyclodextrin (βCD).



Cyclodextrin	R = H or
β-Cyclodextrin	-H
2-Hydroxypropyl-β-cyclodextrin	$-CH_2CHOHCH_3$
Sulfobutylether β-cyclodextrin sodium salt	$-(CH_2)_4SO_3^- Na^+$
Randomly methylated β-cyclodextrin	$-CH_3$
Branched β-cyclodextrin	Glucosyl or maltosyl group

Figure 2. The structure of β-cyclodextrin and some of its derivatives.

Table I. Natural cyclodextrins and some of their derivatives that can be found in marketed pharmaceutical products.

Cyclodextrin	Substitution[a]	MW[b]	Solubility in water (mg/ml)[c]	Indicative bulk price[d] (USD/Kg)
α-Cyclodextrin	-	972	145	45
β-Cyclodextrin (βCD)	-	1135	18.5	5
2-Hydroxypropyl-β-cyclodextrin	0.65	1400	>600	300
Randomly methylated β-cyclodextrin	1.8	1312	>500	350
β-CD sulfobutyl ether sodium salt	0.9	2163	>500	-
γ-Cyclodextrin	-	1297	232	80
2-Hydroxypropyl-γ-cyclodextrin	0.6	1576	>500	400

[a] Average number of substituents per glucopyranose repeat unit.
[b] MW in Daltons.
[c] Solubility in pure water at approx. 25°C.
[d] Approximate bulk price given as the price of one kilogram in US dollars.

The natural α- and β-cyclodextrin, unlike γ-cyclodextrin, cannot be hydrolyzed by human salivary and pancreatic amylases. However, both α- and β-cyclodextrin can be fermented by the intestinal microflora. Cyclodextrins are both large (MW ranging from almost 1000 to over 2000 Daltons) and hydrophilic with a significant number of H-donors and acceptors and, thus, are not absorbed from the gastrointestinal tract in their intact form. Hydrophilic cyclodextrins are considered non-toxic at low to moderate oral dosages. Lipophilic cyclodextrin derivatives, such as the methylated cyclodextrins, are to some extent absorbed from the gastrointestinal tract in to the systemic circulation and have been shown to be toxic after parenteral administration. Presently, oral administration of methylated β-cyclodextrin is limited by its potential toxicity. About 30 different pharmaceutical products containing cyclodextrins are now on the market worldwide. Some of these products are listed in Table II. In the pharmaceutical industry, cyclodextrins have mainly been used as complexing agents to increase the aqueous solubility of poorly water-soluble drugs, and to increase their bioavailability and stability. In addition, cyclodextrins can be used to reduce or prevent gastrointestinal and ocular irritation, reduce or eliminate unpleasant smells or tastes, prevent drug-drug or drug-additive interactions, or to convert oils and liquid drugs into microcrystalline or amorphous powders.

Table II. Regulatory status of the natural cyclodextrins (2004).

	Food Approval			**Pharmacopoeia Monographs**		
	US	**Europe**	**Japan**	**USP/NF**	**Ph.Eur.**	**JP**
αCD	In Preparation	Planned	Yes	No	Yes	Yes
βCD	GRAS	Food Additive	Yes	Yes	Yes	Yes
γCD	GRAS	Pending	Yes	No	In Progress	Yes

The regulatory status of cyclodextrins is evolving. α-Cyclodextrin and β-cyclodextrin are listed in a number of pharmacopoeia sources including the US Pharmacopoeia, European Pharmacopoeia and Japanese Pharmacopoeia. γ-Cyclodextrin will soon be included in the US Pharmacopoeia and subsequently in the European Pharmacopoeia as well. A monograph for 2-hydroxypropyl-β-cyclodextrin has recently appeared in both the European Pharmacopoeia (4^{th} edition (suppl. 4.6) and 5^{th} edition) and in the USP28/NF23. Other derivatives are not yet compendial but efforts are underway for their inclusion. β-Cyclodextrin and γ-cyclodextrin are also listed in the generally regarded as safe (GRAS) list of the FDA for use as a food additive. Cyclodextrins are relatively new from a regulatory point of view and as such policies on their use is still not standardized. Consensus seems to be building among regulators that cyclodextrins are excipients and not part

of the drug substance although various opinion have been given and interpretation related to this point can be division and product-specific.

Table III. Examples of marketed products containing cyclodextrin.

Drug	Administration route	Trade name	Market
α-Cyclodextrin			
Alprostadil (PGE_1)	IV	Prostavastin	Europe, Japan, USA
Cefotiam hexetil HCl	Oral	Pansporin T	Japan
β-Cyclodextrin			
Benexate HCl	Oral	Ulgut, Lonmiel	Japan
Dexamethasone	Dermal	Glymesason	Japan
Iodine	Topical	Mena-Gargle	Japan
Nicotine	Sublingual	Nicorette	Europe
Nimesulide	Oral	Nimedex, Mesulid	Europe
Nitroglycerin	Sublingual	Nitropen	Japan
Omeprazol	Oral	Omebeta	Europe
PGE_2	Sublingual	Prostarmon E	Japan
Piroxicam	Oral	Brexin	Europe
Tiaprofenic acid	Oral	Surgamyl	Europe
2-Hydroxypropyl-β-cyclodextrin			
Cisapride	Rectal	Propulsid	Europe
Hydrocortisone	Buccal	Dexocort	Europe
Indomethacin	Eye drops	Indocid	Europe
Itraconazole	Oral, IV	Sporanox	Europe, USA
Mitomycin	IV	Mitozytrex	USA
Randomly methylated β-cyclodextrin			
17β-Estradiol	Nasal spray	Aerodiol	Europe
Chloramphenicol	Eye drops	Clorocil	Europe
Sulfobutylether β-cyclodextrin			
Voriconazole	IV	Vfend	Europe, USA
Ziprasidone maleate	IM	Geodon, Zeldox	Europe, USA
2-Hydroxypropyl-γ-cyclodextrin			
Diclofenac sodium	Eye drops	Voltaren	Europe

Complex formation and drug solubility

In aqueous solutions cyclodextrins are able to form inclusion complexes with many drugs by taking up a drug molecule, or more frequently some lipophilic moiety of the molecule, into the central cavity. No covalent bonds are formed or broken during the complex formation and drug molecules in the complex are in rapid equilibrium with free molecules in the solution. The driving forces for the complex formation include release of enthalpy-rich water molecules from the cavity, electrostatic interactions, van der Waals interactions, hydrophobic interactions, hydrogen bonding, release of conformational strain and charge-transfer interactions. The physicochemical properties of free drug molecules are different from those bound to the cyclodextrin molecules. Likewise, the physicochemical properties of free cyclodextrin molecules are different from those in the complex. In theory, any methodology that can be used to observe these changes in additive physicochemical properties may be utilized to determine the stoichiometry of the complexes formed and the numerical values of their stability constants. These include changes in solubility, changes in chemical reactivity, changes in UV/VIS absorbance, changes in fluorescence, NMR chemical shifts, changes in drug retention (e.g. in liquid chromatography), changes in pKa values, potentiometric measurements, changes in chemical stability and effects on drug permeability through artificial membranes. Furthermore, since complexation will influence the physicochemical properties of the aqueous complexation media, methods that monitor these media changes can be applied to study the complexation. For example, measurements of conductivity changes, determinations of freezing point depression, viscosity measurements and calorimetric titrations. However, only few of these methods can be applied to obtain structural information on drug/cyclodextrin complexes.

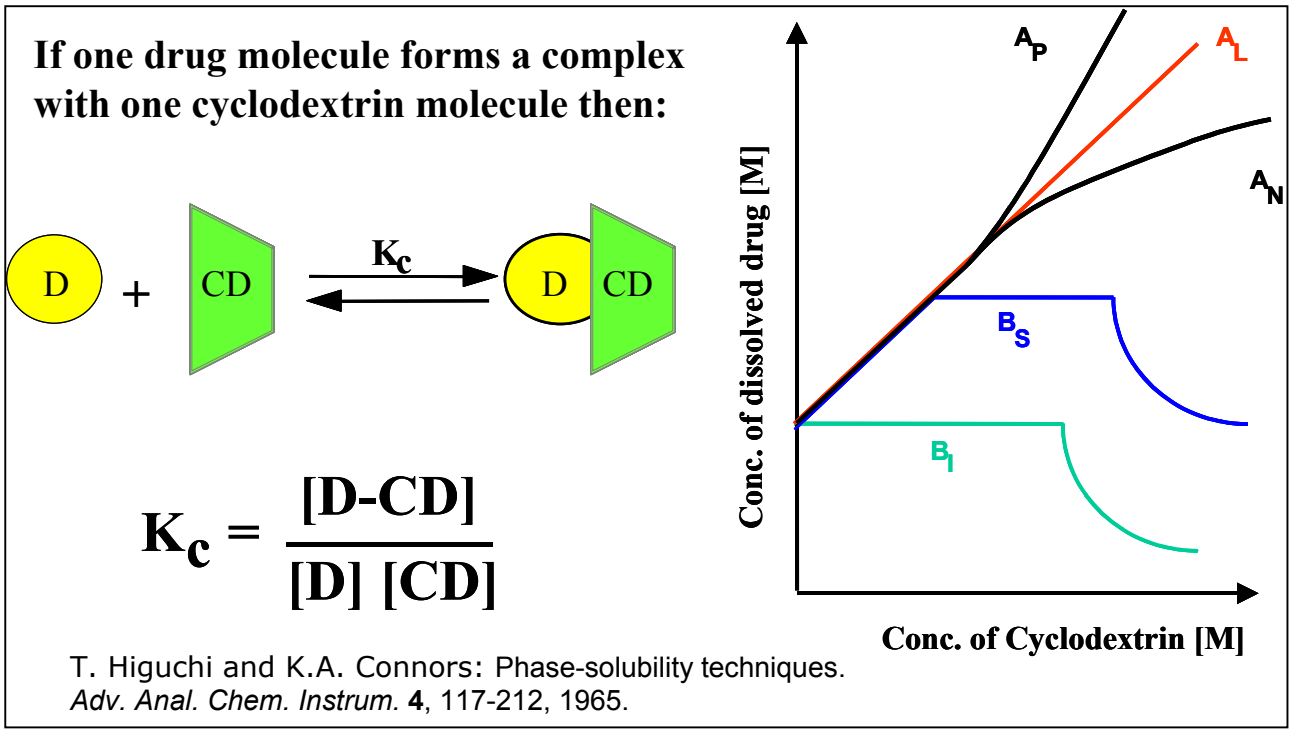


Figure 3. Phase-solubility relationships.

Higuchi and Connors have classified complexes based their effect on substrate solubility as indicated by phase-solubility profiles (Figure 3). A-type phase-solubility profiles are obtained when the solubility of the substrate (i.e. drug) increases with increasing ligand (i.e. cyclodextrin) concentration. When the complex is first order with respect to ligand and first or higher order with respect to substrate then A_L-type phase-solubility profiles is obtained. If the complex is first order with respect to the substrate but second or higher order with respect to the ligand then A_P-type phase-solubility profiles is obtained. A_N-type phase-solubility profiles can be difficult to interpret. B-type phase-solubility profiles indicate formation of complexes with limited solubility in the aqueous complexation medium. In general, the water-soluble cyclodextrin derivatives form A-type phase-solubility profiles while the less soluble natural cyclodextrins frequently form B-type profiles. Most drug/cyclodextrin complexes are thought to be inclusion complexes but cyclodextrins are also known to form non-inclusion complexes and complex aggregates capable to dissolve drugs through micelle-like structures. The phase-solubility profiles do not verify formation of inclusion complexes. They only describe how the increasing cyclodextrin concentration influences drug solubility. The most common type of cyclodextrin complexes is the 1:1 drug/cyclodextrin complex (D/CD) where one drug molecule (D) forms a complex with one cyclodextrin molecule (CD):

$$D + CD \underset{}{\overset{K_{1:1}}{\rightleftharpoons}} D/CD \qquad (1)$$

Under such conditions an A_L-type phase-solubility diagram, with slope less than unity, would be observed and the stability constant ($K_{1:1}$) of the complex can be calculated from the slope and the intrinsic solubility (S_0) of the drug in the aqueous complexation media (i.e. drug solubility when no cyclodextrin is present):

$$K_{1:1} = \frac{Slope}{S_0(1 - Slope)} \qquad (2)$$

The value of $K_{1:1}$ is most often between 50 and 2000 M^{-1} with a mean value of 129, 490 and 355 M^{-1} for α-, β- and γ-cyclodextrin, respectively (Connors KA. The stability of cyclodextrin complexes in solution. *Chem. Rev.* **97**: 1325-1357 (1997)). For 1:1 drug/cyclodextrin complexes the complexation efficiency (CE) can be calculated from the slope of the phase-solubility diagram:

$$CE = \frac{[D/CD]}{[CD]} = S_0 \cdot K_{1:1} = \frac{slope}{(1 - slope))} \qquad (3)$$

When selecting cyclodextrin or complexation conditions during formulation work it can frequently be more convenient to compare the CE than $K_{1:1}$ values. The most common stoichiometry of higher order drug/cyclodextrin complexes is the 1:2

drug/cyclodextrin complex resulting in A_P-type phase-solubility diagram. Consecutive complexation is assumed where the 1:2 complex is formed when one additional cyclodextrin molecule forms a complex with an existing 1:1 complex:

$$D/CD + CD \underset{}{\overset{K_{1:2}}{\rightleftharpoons}} D/CD_2 \qquad (4)$$

The stoichiometry of the system can be probed by curve fitting of the diagram with a quadratic model:

$$S_{tot} = S_0 + K_{1:1} S_0 [CD] + K_{1:1} K_{1:2} S_0 [CD]^2 \qquad (5)$$

Here [CD] represents the concentration of free cyclodextrin but it is customary to plot the total amount of dissolved drug (S_{tot}) against the total amount of cyclodextrin in solution ($[CD]_{tot}$) assuming that the extent of complexation is low (i.e. [CD] ~ $[CD]_{tot}$). The value of $K_{1:2}$ is frequently between 10 and 500 M^{-1}, or significantly lower than that of $K_{1:1}$.

Various methods can be applied to prepare drug/cyclodextrin complexes, including the solution method, the co-precipitation method, neutralization method, the slurry method, the kneading method, and the grinding method (A. R. Hedges, Industrial applications of cyclodextrins. *Chem. Rev.* **98**: 2035-2044 (1998)). In most cases presence of at least some water is essential for successful complex formation. In solution, cyclodextrin complexes are usually prepared by addition of excess amount of drug to an aqueous cyclodextrin solution. The suspension formed is equilibrated at the desired temperature (which may require periods of up to one week) and then filtered or centrifuged to form clear drug/cyclodextrin complex solution. For preparation of solid complexes, the water is removed from the aqueous drug/cyclodextrin solution by evaporation (e.g. spay-drying) or sublimation (e.g. lyophilization).

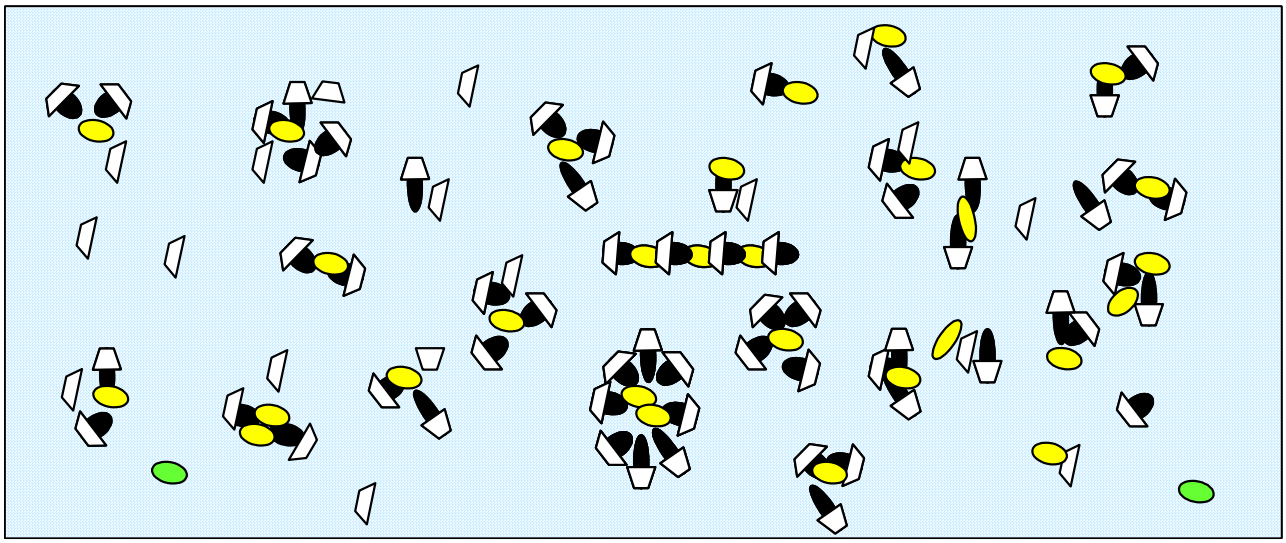
For variety of reasons, such as isotonicity of parenteral formulations and formulation bulk of solid dosage forms, it is important to include as little cyclodextrin as possible in a pharmaceutical formulation. Various methods have been applied to enhance the complexation efficacy (T. Loftsson, M. Másson, J.F. Sigurjónsdóttir, Methods to enhance the complexation efficiency of cyclodextrins. *S.T.P. Pharma Sci.* **9**: 237-242. (1999)). These include addition of polymers to the complexation media, drug ionization and salt formation, addition of hydroxy carboxylic acids to the complexation media, addition of volatile acids or bases to the complexation media, addition of organic salts, and addition of cosolvents (Table IV). However, even under the best conditions, cyclodextrin complexation will result in over four-fold increase in the formulation bulk of solid dosage forms.

Table IV. Some methods that can be applied to enhance the complexation efficiency.

Effect	**Consequences**
Dug ionization	Unionized drugs do usually form more stable complexes than their ionic counterparts. However, ionization of a drug increases its apparent intrinsic solubility resulting in enhanced complexation.
Salt formation	It is sometimes possible to enhance the apparent intrinsic solubility of a drug through salt formation.
Complex-in-complex	It is sometime possible to increase the apparent intrinsic solubility of a drug through formation of metal complexes.
The acid/base ternary complexes	It has been shown that certain organic hydroxy acids (such as citric acid) and certain organic bases are able to enhance the complexation efficiency by formation of ternary drug/cyclodextrin/acid or base complexes.
Polymer complexes	Water-soluble polymers form a ternary complex with drug/cyclodextrin complexes increasing the observed stability constant of the drug/cyclodextrin complex. This observed increase in the value of the constant increases the complexation efficiency.
Solubilization of cyclodextrin aggregates	Organic cations and anions are known to solubilize uncharged drug/cyclodextrin complexes that have limited aqueous solubility. This will enhance the complexation efficiency during preparation of, for example, solid drug/cyclodextrin complex powder.
Combination of two or more methods	Frequently the complexation efficiency can be enhanced even further my combining two or more of the above mentioned methods. For example drug ionization and the polymer method, or solubilization of the cyclodextrin aggregates by adding both polymers and cations or anions to the aqueous complexation medium.

Non-conventional cyclodextrin complexes

It has generally been assumed that the mechanism whereby cyclodextrin exert their effects, especially their augmentation of solubility, is via the formation of non-covalent, dynamic inclusion complexes. This is a model, which regards drug-cyclodextrin interactions as discrete phenomenon and ignores the possible interaction of these complexes with one another. It is becoming increasingly apparent that such assumptions may not be universally applicable or all encompassing. Specifically, there is a growing body of evidence that supports the important contribution of non-inclusion-based aspects for drug solubilization by cyclodextrins including surfactant-like effects and molecular aggregation. This short review attempts to assess the available literature for areas where such non-inclusion mechanisms are apparent and tries to interpret these in the context of a broader working theory as to how cyclodextrin exert their beneficial effects.



Free drug molecule

Drug in a non-inclusion complex

Drug in an inclusion complex

“Empty” cyclodextrin molecule

Drug/cyclodextrin inclusion complex

T. Loftsson, A. Magnúsdóttir, M. Másson and J. F. Sigurjónsdóttir, „Self-association and cyclodextrin solubilization of drugs“, *J. Pharm. Sci.* **91**, 2307-2316 (2002).

T. Loftsson, M. Másson and M. E. Brewster, „Self-Association of cyclodextrins and cyclodextrin complexes”, *J. Pharm. Sci.* **93**, 1091-1099 (2004).

Expert Opin. Drug Deliv. 2, 335-351 (2005).

Review

Expert Opinion

1. Introduction
2. Cyclodextrins
3. Formulation with cyclodextrins
4. Expert opinion and conclusion

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Cyclodextrins in drug delivery

Thorsteinn Loftsson†, Pekka Jarho, Már Másson & Tomi Järvinen
†*University of Iceland, Faculty of Pharmacy, Hagi, Hofsvallagata 53, IS-107, Reykjavik,Iceland*

Cyclodextrins are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. Cyclodextrin molecules are relatively large with a number of hydrogen donors and acceptors and, thus, in general they do not permeate lipophilic membranes. In the pharmaceutical industry cyclodextrins have mainly been used as complexing agents to increase aqueous solubility of poorly soluble drugs, and to increase their bioavailability and stability. Studies in both humans and animals have shown that cyclodextrins can be used to improve drug delivery from almost any type of drug formulation. However, addition of cyclodextrins to existing formulations without further optimisation will seldom result in acceptable outcome. Currently there are worldwide about 30 different pharmaceutical products containing drug/cyclodextrin complexes on the market.

Keywords: absorption, bioavailability, cyclodextrin, drug delivery, formulation, solubilisation, stabilisation

Expert Opin. Drug Deliv. (2005) 2(2):xxx–xxx

1. Introduction

All drugs must possess some degree of aqueous solubility to be pharmacologically active, and most drugs need to be lipophilic to be able to permeate biological membranes via passive diffusion. How water-soluble a given drug needs to be is determined by its potency (i.e., the dosage size) and type of formulation. For example, in an aqueous eye drop formulation the dose should be soluble in < 50 µl (i.e., one drop) of water, but in a parenteral formulation the dose should preferably be soluble in < 5 ml of water, corresponding to solubility > ~ 15 mg/ml for a medium potent drug (i.e., dose of about 1 mg/kg) [1]. Oral absorption of drugs with solubilities < 0.1 mg/ml is likely to be dissolution limited [2]. On the other hand, if a drug is too water soluble (and/or too hydrophilic) the dissolved drug molecule will have little tendency to partition from the aqueous exterior into a lipophilic biomembrane (e.g., the eye cornea or gastrointestinal mucosa) and then to permeate the membrane. High-throughput screening approaches to drug development have led to an increasing number of lipophilic water-insoluble drug candidates [3] or drugs whose clinical usefulness is hampered by their insolubility in water. These drugs are classified as Class II (i.e., poorly soluble/highly permeable) or Class IV (i.e., poorly soluble/poorly permeable) drugs according to the Biopharmaceutics Classification System [4]. In general, formulation techniques that increase the apparent aqueous solubility of Class II and Class IV drugs without decreasing their lipophilicity will enhance their absorption through biological membranes. These techniques include particle size reduction, salt formation, solid dispersion, melt extrusion, spray drying, and complexation, as well as drug solutions in microemulsions, liposomes, and non-aqueous solvents. The following is a review of cyclodextrins and their place in drug delivery.

2. Cyclodextrins

Cyclodextrins are natural cyclic oligosaccharides that were discovered > 100 years ago [5], but only recently did highly purified cyclodextrins become available as pharmaceutical excipients. Worldwide about 30 different pharmaceutical products

Table 1. Some examples of marketed products containing cyclodextrin.

Drug	Formulation	Trade name	Company
α-Cyclodextrin			
Alprostadil (PGE_1)	IV solution	Prostavasin	Ono (Japan)
Cefotiam hexetil HCl	Oral tablet	Pansporin T	Takeda (Japan)
β-Cyclodextrin			
Benexate HCl	Oral capsule	Ulgut	Teikoku Kagaku Sangyou (Japan)
Dexamethasone	Dermal ointment	Glymesason	Fujinaga (Japan)
Nicotine	Sublingual tablet	Nicorette	Pharmacia (Sweden)
Nitroglycerin	Sublingual tablet	Nitropen	Nihon Kayaku (Japan)
Piroxicam	Oral tablet	Brexin	Chiesi (Italy)
Tiaprofenic acid	Oral tablet	Surgamyl	Roussel-Maestrelli (Italy)
2-Hydroxypropyl-β-cyclodextrin			
Cisapride	Suppository	Propulsid	Janssen (Belgium)
Indomethacin	Eye drop solution	Indocid	Chauvin (France)
Itraconazole	Oral and IV solutions	Sporanox	Janssen (Belgium)
Mitomycin	IV solution	Mitozytrex MitoExtra	SuperGen (USA) Novartis (Switzerland)
Randomly methylated β-cyclodextrin			
17β-Oestradiol	Nasal spray	Aerodiol	Servier (France)
Chloramphenicol	Eye drop solution	Clorocil	Oftalder (Portugal)
Sulfobutylether β-cyclodextrin			
Voriconazole	IV solution	Vfend	Pfizer (USA)
Ziprasidone maleate	IM solution	Geodon, Zeldox	Pfizer (USA)
2-Hydroxypropyl-γ-cyclodextrin			
Diclofenac sodium	Eye drop solution	Voltaren ophtha	Novartis (Switzerland)

containing cyclodextrins are on the market (**Table 1**). In the pharmaceutical industry cyclodextrins have mainly been used as complexing agents to increase aqueous solubility of poorly soluble drugs, and to increase their bioavailability and stability. In addition, cyclodextrins can, for example, be used to reduce gastrointestinal drug irritation, convert liquid drugs into microcrystalline or amorphous powder, and prevent drug–drug and drug–excipient interactions. A number of books and review articles have been published on the pharmaceutical applications of cyclodextrins [6-18].

2.1 Structure and properties

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. They consist of (α-1,4-)-linked α-D-glucopyranose units with a lipophilic central cavity (**Figure 1**). Due to the chair formation of the glucopyranose units, cyclodextrin molecules are shaped like cones with secondary hydroxy groups extending from the wider edge and the primary groups from the narrow edge. This gives cyclodextrin molecules a hydrophilic outer surface, whereas the lipophilicity of their central cavity is comparable to an aqueous ethanolic solution [8]. The most common natural cyclodextrins consist of six (α-cyclodextrin), seven (β-cyclodextrin) and eight (γ-cyclodextrin) glucopyranose units. Although the natural cyclodextrins and their complexes are hydrophilic, their aqueous solubility is rather limited, especially that of β-cyclodextrin. This is thought to be due to relatively strong binding of the cyclodextrin molecules in the crystal state (i.e., relatively high crystal lattice energy) [9]. Random substitution of the hydroxy groups, even by hydrophobic

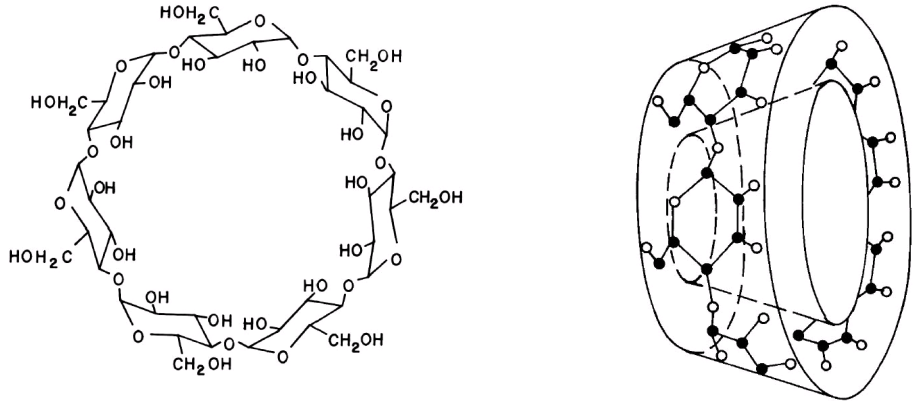


Figure 1. The chemical structure and the conical shape of the β-cyclodextrin molecule. Used with permission from Journal of Pharmaceutical Sciences © (1996) Wiley-Liss, Inc., A Wiley Company [9].

Table 2. Cyclodextrins that can be found in marketed pharmaceutical products.

Cyclodextrin	Substitution*	MW (Da)	Solubility in water (mg/ml)‡	Applications
α-Cyclodextrin	-	972	145	Oral, parenteral, topical
β-Cyclodextrin	-	1135	18.5	Oral, topical
2-Hydroxypropyl-β-cyclodextrin	0.65	1400	> 600	Oral, parenteral, topical
Randomly methylated β-cyclodextrin	1.8	1312	> 500	Oral§, topical
β-Cyclodextrin sulfobutyl ether sodium salt	0.9	2163	> 500	Oral, parenteral, topical
γ-Cyclodextrin	-	1297	232	Oral, parenteral§, topical
2-Hydroxypropyl-γ-cyclodextrin	0.6	1576	> 500	Oral, parenteral, topical

*Average number of substituents per glucopyranose repeat unit. ‡Solubility in pure water at ~ 25°C. §In very limited amounts.
MW: Molecular weight.

moieties such as methoxy functions, will result in dramatic improvements in their solubility (**Table 2**). The main reason for the solubility enhancement is that the random substitution transforms the crystalline cyclodextrins into amorphous mixtures of isomeric derivatives. Cyclodextrin derivatives of pharmaceutical interest include the hydroxypropyl derivatives of β- and γ-cyclodextrin, the randomly methylated β-cyclodextrin, sulfobutylether β-cyclodextrin, and the so-called branched cyclodextrins such as glucosyl-β-cyclodextrin.

Cyclodextrin molecules are relatively large (molecular weight ranging from almost 1000 to > 2000 Da) with a large number of hydrogen donors and acceptors, and are consequently poorly absorbed through biological membranes. The natural α- and β-cyclodextrin, unlike γ-cyclodextrin, cannot be hydrolysed by human salivary and pancreatic amylases [19,20], but all three are subjected to fermentation by the intestinal microflora. Hydrophilic cyclodextrins are non-toxic at low to moderate oral dosages [11,15]. The natural cyclodextrins and their derivatives are used in topical and oral formulations, but only α-cyclodextrin and the hydrophilic derivatives of β- and γ-cyclodextrin can be used in parenteral formulations. γ-Cyclodextrin forms visible aggregates in aqueous solutions and, thus, is not well suited for parenteral formulations [21]. Due to its nephrotoxicity, β-cyclodextrin cannot be used in parenteral formulations. Lipophilic cyclodextrin derivatives, such as the methylated cyclodextrins, are to some extent absorbed from the gastrointestinal tract into the systemic circulation and have been shown to be toxic after parenteral administration [11]. Presently, oral administration of methylated β-cyclodextrin is limited by its potential toxicity. Cyclodextrin monographs can be found in several Pharmacopoeias. For example, α-cyclodextrin and β-cyclodextrin are listed in the US Pharmacopeia, European Pharmacopeia and the Japanese Pharmacopeia. γ-Cyclodextrin will soon be included in the US Pharmacopeia and subsequently in the European Pharmacopeia as well. A monograph for 2-hydroxypropyl-β-cyclodextrin has recently appeared in the European Pharmacopeia. β-Cyclodextrin and γ-cyclodextrin are also listed in the 'generally regarded as safe' list of the FDA for use as food additives.

2.2 Complex formation and drug solubility

In aqueous solutions cyclodextrins are able to form inclusion complexes with many drugs by taking up a drug molecule, or more frequently some lipophilic moiety of the molecule, into the central cavity. No covalent bonds are formed or broken during the complex formation, and drug molecules in the

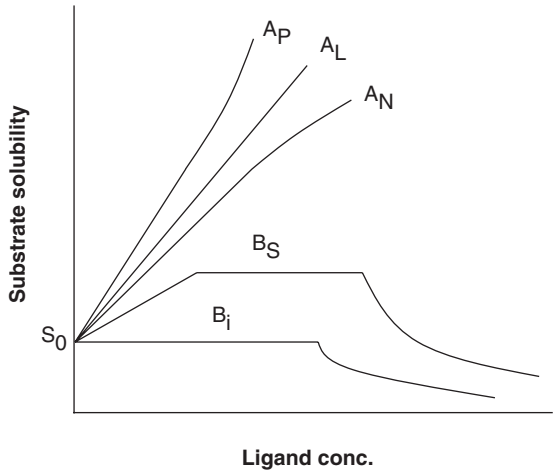


Figure 2. Phase-solubility profiles and classification of complexes according to Higuchi and Connors [26]. S_0 is the intrinsic solubility of the substrate (the drug) in the aqueous complexation medium when no ligand (cyclodextrin) is present.

complex are in rapid equilibrium with free molecules in the solution. The driving forces for the complex formation include release of enthalpy-rich water molecules from the cavity (i.e., water molecules that cannot have a full compliment of hydrogen bonds), electrostatic interactions, van der Waals' interactions, hydrophobic interactions, hydrogen bonding, release of conformational strain and charge-transfer interactions [9,22]. The physicochemical properties of free drug molecules are different from those bound to the cyclodextrin molecules. Likewise, the physicochemical properties of free cyclodextrin molecules are different from those in the complex. In theory, any methodology that can be used to observe these changes in additive physicochemical properties may be utilised to determine the stoichiometry of the complexes formed and the numerical values of their stability constants [23-25]. These include changes in solubility, chemical reactivity, UV/VIS absorbance, fluorescence, drug retention (e.g., in liquid chromatography), pKa values, potentiometric measurements and chemical stability, nuclear magnetic resonance (NMR) chemical shifts and effects on drug permeability through artificial membranes. Furthermore, because complexation will influence the physicochemical properties of the aqueous complexation media, methods that monitor these media changes can be applied to study the complexation; for example, measurements of conductivity changes, determinations of freezing point depression, viscosity measurements and calorimetric titrations. However, only few of these methods can be applied to obtain structural information on drug/cyclodextrin complexes.

Higuchi and Connors [26] have classified complexes based their effect on substrate solubility as indicated by phase-solubility profiles (**Figure 2**). A-type phase-solubility profiles are obtained when the solubility of the substrate (i.e., drug) increases with increasing ligand (i.e., cyclodextrin) concentration. When the complex is first order with respect to ligand and first or higher order with respect to substrate then A_L-type phase-solubility profiles are obtained. If the complex is first order with respect to the substrate, but second or higher order with respect to the ligand then A_P-type phase-solubility profiles are obtained. A_N-type phase-solubility profiles can be difficult to interpret. The negative deviation from linearity may be associated with cyclodextrin-induced changes in the dielectric constant of the aqueous complexation media, changes in complex solubility or self-association of cyclodextrin molecules [24]. B-type phase-solubility profiles indicate formation of complexes with limited solubility in the aqueous complexation medium. In general, the water-soluble cyclodextrin derivatives form A-type phase-solubility profiles, whereas the less soluble natural cyclodextrins frequently form B-type profiles. Most drug/cyclodextrin complexes are thought to be inclusion complexes, but cyclodextrins are also known to form non-inclusion complexes and complex aggregates capable of dissolving drugs through micelle-like structures [27,28]. The phase-solubility profiles do not verify formation of inclusion complexes. They only describe how the increasing cyclodextrin concentration influences drug solubility. To distinguish between inclusion and non-inclusion complexes, experimental results from phase-solubility studies have to be compared with other experimental results from, for example, UV/VIS, fluorescence and NMR studies [27,28]. The most common type of cyclodextrin complex is the 1:1 drug/cyclodextrin complex (D/CD) in which one drug molecule (D) forms a complex with one cyclodextrin molecule (CD):

$$(1) \quad D + CD \overset{K_{1:1}}{\leftrightarrow} D/CD$$

Under such conditions an A_L-type phase-solubility diagram, with slope less than unity, would be observed, and the stability constant ($K_{1:1}$) of the complex can be calculated from the slope and the intrinsic solubility (S_0) of the drug in the aqueous complexation media (i.e., drug solubility when no cyclodextrin is present):

$$(2) \quad K_{1:1} = Slope/[S_0(1 - Slope)]$$

The value of $K_{1:1}$ is most often between 50 and 2000 M^{-1} with a mean value of 129, 490 and 355 M^{-1} for α-, β- and γ-cyclodextrin, respectively [12,29-31]. For 1:1 drug/cyclodextrin complexes the complexation efficiency (CE) can be calculated from the slope of the phase-solubility diagram:

$$(3) \quad CE = [D/CD]/[CD] = S_0 \cdot K_{1:1} = Slope/(1 - Slope)$$

When selecting cyclodextrin or complexation conditions during formulation work it can frequently be more convenient to compare the CE than $K_{1:1}$ values. The most common stoichiometry of higher order drug/cyclodextrin complexes is the 1:2 drug/cyclodextrin complex resulting in A_P-type phase-solubility diagram. Consecutive complexation is assumed where the 1:2 complex is formed when one

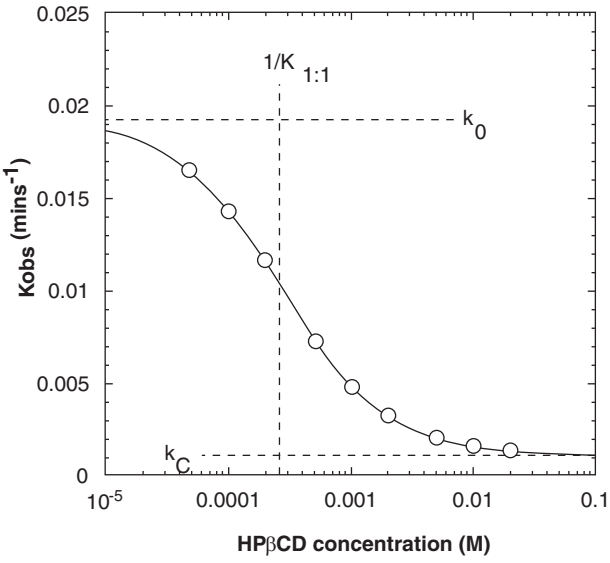


Figure 3. Results from a kinetic study of the effect of cyclodextrin on drug stability. Degradation of chlorambucil in 2-hydroxypropyl-β-cyclodextrin (HPβCD) solution (10 mM NaH_2PO_4/NaOH buffer, pH 7.5, 30 °C).

additional cyclodextrin molecule forms a complex with an existing 1:1 complex [24]:

$$D/CD + CD \overset{K_{1:2}}{\leftrightarrow} D/CD_2 \quad (4)$$

The stoichiometry of the system can be probed by curve fitting of the diagram with a quadratic model:

$$S_{tot} = S_0 + K_{1:1}S_0[CD] + K_{1:1}K_{1:2}S_0[CD]^2 \quad (5)$$

Here [CD] represents the concentration of free cyclodextrin, but it is customary to plot the total amount of dissolved drug (S_{tot}) against the total amount of cyclodextrin in solution ($[CD]_{tot}$), assuming that the extent of complexation is low (i.e., $[CD] \sim [CD]_{tot}$). The value of $K_{1:2}$ is frequently between 10 and 500 M^{-1}, or significantly lower than that of $K_{1:1}$.

Various methods can be applied to prepare drug/cyclodextrin complexes, including the solution method, the co-precipitation method, neutralisation method, the slurry method, the kneading method, and the grinding method [23,32]. In most cases presence of at least some water is essential for successful complex formation. In solution, cyclodextrin complexes are usually prepared by addition of excess amount of drug to an aqueous cyclodextrin solution. The suspension formed is equilibrated at the desired temperature (which may require periods of up to 1 week) and then filtered or centrifuged to form clear drug/cyclodextrin complex solution. For preparation of solid complexes, the water is removed from the aqueous drug/cyclodextrin solution by evaporation (e.g., spray drying) or sublimation (e.g., lyophilisation).

For a variety of reasons, such as isotonicity of parenteral formulations and formulation bulk of solid dosage forms, it is important to include as little cyclodextrin as possible in a pharmaceutical formulation. Various methods have been applied to enhance the complexation efficacy [33]. These include addition of polymers to the complexation media [34], drug ionisation and salt formation [35,36], addition of hydroxy carboxylic acids to the complexation media [37], addition of volatile acids or bases to the complexation media [38], addition of organic salts [39], and addition of cosolvents [40]. However, even under the best conditions, cyclodextrin complexation will result in over fourfold increase in the formulation bulk of solid dosage forms [33]. Rao and Stella [31] have shown how the feasibility of using cyclodextrins in dosage forms can be calculated from few simple experiments.

2.3 Drug stability

Stability issues can limit the feasibility of a pharmaceutical formulation. This is especially true for aqueous formulations of drugs that are prone to hydrolysis or oxidation. The reaction rates can be affected by inclusion of the drug, and especially inclusion of its chemically labile moiety, into the cyclodextrin cavity.

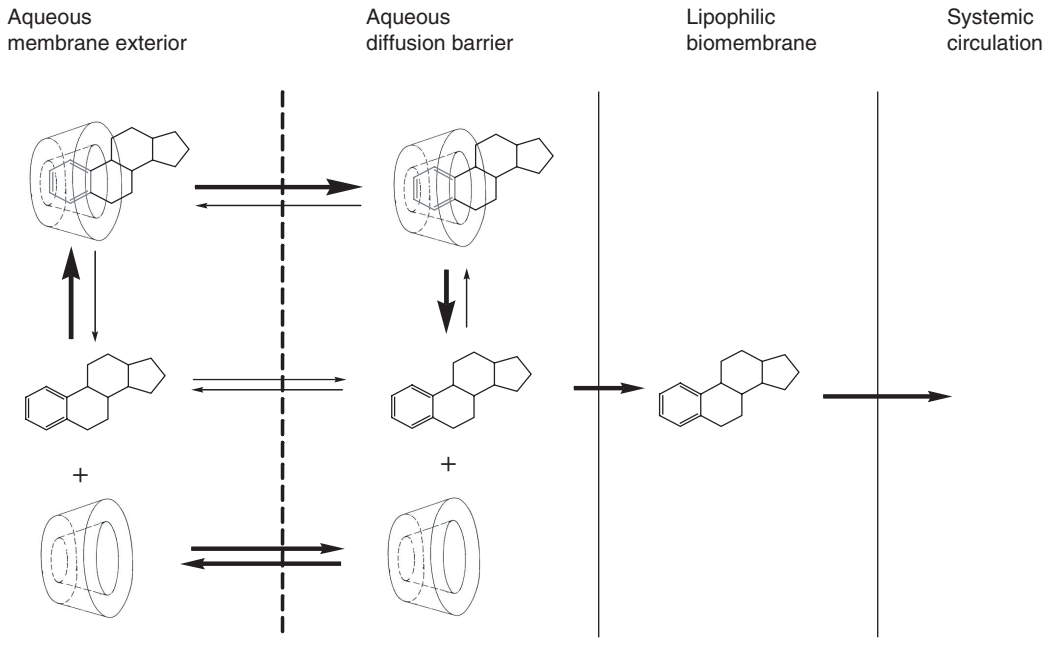
In cyclodextrin solutions the observed degradation rate for a chemically unstable compound, forming 1:1 complex, will be the weighted average of the degradation rates of the free drug and drug in cyclodextrin complex. In first-order and pseudo-first-order reactions, such as hydrolysis or oxidation, the stabilising (or catalytic) effect will depend on three parameters: the cyclodextrin concentration, the stability constant of the complex, and the degradation rate constant for the drug degradation within the cyclodextrin cavity (k_C). These parameters can be determined by fitting degradation data obtained at several cyclodextrin concentrations to Equation 7:

$$\begin{array}{ccc} & \overset{K_{1:1}}{} & \\ D + CD & \leftrightarrow & D/CD \\ k_0 \downarrow & & \downarrow k_c \\ \text{Degradation products} & & \end{array} \quad (6)$$

$$k_{obs} = (k_0 + k_c K_{1:1}[CD]) / (1 + K_{1:1}[CD]) \quad (7)$$

Where k_{obs} is observed rate constant for the reaction and k_0 is the rate constant for the reaction in pure aqueous solution. An example of such kinetic study, of chlorambucil degradation in aqueous 2-hydroxypropyl-β-cyclodextrin solution, is shown in **Figure 3** [41]. Through nonlinear fitting of Equation 7, the values for $K_{1:1}$ (3836 ± 89 M^{-1}, mean ± standard deviation), k_o and k_c can be obtained (k_o/k_c = 17). From **Figure 3** it can be seen that 50% of the maximum stabilising effect is obtained when the cyclodextrin concentration is equal to $1/K_{1:1}$. In many of the older publications the values of k_C and $K_{1:1}$ were obtained by linear fitting to Equation 8 [42]:

Table 3. The effect of cyclodextrin complexation on drug bioavailability after non-parenteral administration.



FDA class*	Drug properties		RDS to drug absorption¶	Effect of cyclodextrin complexation
	***Aqueous solubility*‡**	***Permeability*§**		
I	Highly soluble	Highly permeable	(Good bioavailability)	Can decrease absorption
II	Poorly soluble	Highly permeable	Aqueous diffusion	Can enhance absorption
III	Highly soluble	Poorly permeable	Membrane permeation	Can decrease absorption
IV	Poorly soluble	Poorly permeable	Aqueous diffusion and membrane permeation	Can enhance absorption

*FDA Biopharmaceutics Classification System of orally administered drugs. ‡Intrinsic solubility of the drug in the aqueous membrane exterior. §Passive drug permeation through lipophilic biomembrane such as the gastrointestinal mucosa. ¶RDS of drug delivery from the aqueous exterior into the body.
FDA: Food and Drug Association; RDS: Rate determining step.

$$(8) \quad 1/(k_0 - k_{obs}) = 1/(k_0 - k_c) + 1/K_{1:1}\,(k_0 - k_c)\,1/[CD]$$

However, because the nonlinear method is less sensitive to experimental errors than the linear one, it frequently gives more accurate results [43,44]. Many studies have shown that stability of chemically labile drugs and compounds such as steroid esters [45], alkylating anticancer agents [46-49], prostaglandins [50-52], prodrugs [53,54] and various other drug compounds [9,42] can be improved through formulation with cyclodextrins. Most of these studies have been focused on drug stability in aqueous solutions, but cyclodextrins have also been shown to stabilise drugs in solid dosage forms [55,56]. The photochemistry of cyclodextrin inclusion complexes has been studied [57] and the effect of complexation on photostability of drugs has been investigated [58]. These studies have shown that cyclodextrin complexation can affect the light absorption properties of the inclusion compound and primary and secondary photochemical reactions, and that the effect can be either stabilising or destabilising. In general, these effects are modest and may depend on the type of cyclodextrin used and on the experimental conditions, such as the irradiation wavelength. Finally, cyclodextrins can also increase physical stability of drugs. For example, evaporation of volatile compounds can be significantly reduced through complex formation [32], and cyclodextrins have been used to prevent aggregation and to reduce denaturation in peptide and protein formulations [59-61].

Cyclodextrins can sometimes have a destabilising effect on drugs through direct catalysis or, for example, by enhancing drug solubility in aqueous drug suspensions [9,42]. Frequently, the catalytic effect is associated with deprotonisation of the hydroxy groups located at the rim of the cyclodextrin cavity [62-64]. In this way cyclodextrins behave like carbohydrates and other polyhydric alcohols with adjacent hydroxy groups [63]. In this case the catalytic effect will mainly be observed under basic conditions and will increase with increasing pH.

2.4 Drug delivery through biological membranes

The chemical structure of cyclodextrins (i.e., the large number of hydrogen donors and acceptors), their molecular weight (i.e., > 970 Da) and their very low octanol/water partition coefficient (approximately log $P_{o/w}$ between -3 and 0.00 [65]) are all characteristics of compounds that do not readily permeate biological membranes [4,66]. In fact, experiments have shown that only negligible amounts of hydrophilic cyclodextrins and drug/cyclodextrin complexes are able to permeate lipophilic membranes such as skin and gastrointestinal mucosa [11,67]. Only the free form of the drug, which is in equilibrium with the drug/cyclodextrin complex, is capable of penetrating lipophilic membranes [68]. Cyclodextrins are able to extract lipophilic components from biomembranes such as stratum corneum [69,70], but both pre- and postapplication of hydrophilic cyclodextrins does not affect, for example, the skin barrier [71,72]. Cyclodextrins do not, in general, enhance permeability of hydrophilic water-soluble drugs through lipophilic biological membranes [73,74], and numerous studies have shown that excess cyclodextrin will reduce drug permeability through biological membranes [73]. The physicochemical properties of the drug (e.g., its solubility in water), the composition of the drug formulation (e.g., aqueous or non-aqueous) and physiological composition of the membrane barrier (e.g., presence of an aqueous diffusion layer), will determine whether cyclodextrins will enhance or hamper drug delivery through a biological membrane (**Table 3**). Most biological membrane barriers (or biomembranes) are lipophilic with an aqueous exterior, which forms a structured water layer at the membrane surface frequently referred to as unstirred diffusion layer. If drug permeation through the aqueous diffusion layer is the rate-limiting step of drug permeation through the barrier, cyclodextrins can frequently enhance the permeation. However, cyclodextrins are in most cases unable to enhance drug permeation through a lipophilic membrane barrier and excess cyclodextrin (more than is needed to dissolve the drug) will hamper drug permeation through the membrane. In other words, cyclodextrins will enhance drug delivery through aqueous diffusion-controlled barriers, but can hamper drug delivery through lipophilic membrane-controlled barriers [74]. However, there is one exception: lipophilic cyclodextrins, such as the methylated β-cyclodextrins, are able to permeate mucosa and are known to enhance drug delivery through biological membranes, such as through the nasal mucosa, by reducing barrier function of the membranes [75].

Cyclodextrins can, at least in theory, enhance drug bioavailability by stabilisation of drug molecules at the biomembrane surface. For example, cyclodextrins have been shown to prevent insulin aggregation and to enhance insulin stability at the nasal mucosa. It has been suggested that cyclodextrin-enhanced insulin bioavailability after nasal administration is partly due to this stabilising effect [76]. In general, drug stabilisation associated with cyclodextrin complexation plays only a very minor role when it comes to drug delivery through biological membranes. It is their solubilising effect that is usually related to improved drug delivery. However, as cyclodextrins can both enhance and hamper drug delivery through biological membranes it is of utmost importance to optimise cyclodextrin-containing drug formulations with regard to drug delivery from the formulations [73]. Too much or too little cyclodextrin can result in less than optimum drug bioavailability.

3. Formulation with cyclodextrins

It is important for a pharmaceutical formulator to know the advantages and limitations of each excipient used during design of a product. Excipients are selected based on the physicochemical properties of the drug (e.g., solubility, stability etc.), type of delivery (e.g., tablet, parenteral solution etc.) and desired pharmacokinetics (e.g., instant release, sustained release etc.). The following is a brief overview of the use of cyclodextrins in various formulations, with the main emphasis on the effects of cyclodextrins on aqueous solubility and drug permeability through biological membranes. However, cyclodextrins are also able to increase the physical and chemical stability of drugs in the various formulations (i.e., increasing the shelf-life of the pharmaceutical product) as well as to reduce local drug irritation.

3.1 Oral drug delivery

Drug absorption from immediate-release tablets in the gastrointestinal tract consists of a series of rate processes including drug dissolution in the aqueous gastrointestinal fluids, permeation of the drug molecules from the intestinal fluid through an aqueous diffusion layer immediately adjacent to the mucosal surface, and permeation through the mucosa. The effect of cyclodextrins on oral drug absorption can be explained in the context of the Biopharmaceutics Classification System (**Table 4**) [77]. The Biopharmaceutics Classification System categorises drugs according to their aqueous solubility and ability to permeate the intestinal mucosa (**Table 3**). A given drug substance is considered 'highly soluble' when the highest dose strength is soluble in ≤ 250 ml water over a pH range of 1.0 – 7.5, and 'highly permeable' when the extent of oral absorption in humans is determined to be ≥ 90% of an administered dose (in solution). For an immediate-release tablet, ≥ 85% of the labelled amount of drug substance must dissolve within 30 min [2,4,301]. Class I drugs are relatively water soluble and their absolute bioavailability is ≥ 90%. These drugs permeate easily through the aqueous diffusion layer and possess sufficient lipophilicity to partition into and then permeate through the gastrointestinal mucosa. In general, hydrophilic cyclodextrins are not able to improve bioavailability of Class I drugs. However, cyclodextrin can be used to reduce local drug irritation and increase rate of drug absorption. Class II drugs have limited aqueous solubility, resulting in dissolution-rate limited oral absorption. However, once in solution these drugs permeate biological membranes relatively easily, resulting in ≥ 90% absolute bioavailability. Thus, low

Table 4. Some examples of cyclodextrins in oral formulations, tested *in vivo* in humans and/or animals, and the effect of the cyclodextrin complexation on the absolute bioavailability compared to identical cyclodextrin-free formulation.

Drug	Cyclodextrin	Formulation	Species	F_{rel}*	Ref.
Class I					
Piroxicam	βCD	Tablet, capsule and oral suspension	Human, rat, rabbit	≤1.4	[128-131]
Class II					
Carbamazepine	DMβCD	Oral powder and solution, tablet	Rabbit, dog, rat	≤5.6	[132-136]
Digoxin	γCD	Tablet	Dog	5.4	[137]
Glibenclamide	βCD, SBEβCD	Capsule containing powder	Dog, rat	≤6.2	[138, 139]
Miconazole	HPβCD	Aqueous suspension	Rat	2.3	[140]
Phenytoin	E-βCD, GluβCD, MalβCD, SBEβCD, HPβCD	Suspension, capsule containing powder	Rat, Dog	≤5	[141-143]
Spironolactone	βCD, γCD, DMβCD, SBEβCD, HPβCD	Oral solution and powder	Rat, dog	≤3.6	[144-146]
Tolbutamide	βCD, HPβCD	Suspension, oral powder	Rabbit, dog	≤1.5	[147, 148]
α-Tocopheryl nicotinate	DMβCD	Capsule containing powder	Dog	~70	[149]
Class III					
Acyclovir	βCD	Oral suspension	Rat	1.1	[150]
Diphenhydramine HCl	DMβCD, HPβCD	Solution	Rat	≤0.9	[151]
Class IV					
Cyclosporin A	DMβCD	Oral suspension	Rat	4.7	[152, 153]

*F_{rel} (i.e., the AUC of the plasma concentration versus time profile when the cyclodextrin-containing formulation was given divided by the AUC for the formulation containing no cyclodextrin.

AUC: area-under-curve; βCD: β-Cyclodextrin; γCD: γ-Cyclodextrin; DMβCD: Dimethyl-β-cyclodextrin; E-βCD: β-Cyclodextrin epichlorohydrin polymer; F_{rel} : Relative bioavailability; Gluβ CD: Glucosyl-β-cyclodextrin; HPβCD: 2-Hydroxypropyl-β-cyclodextrin; MalβCD: Maltosyl-β-cyclodextrin; SBEβCD: Sulfobutylether-β-cyclodextrin sodium salt.

aqueous solubility hampers their dissolution rate. The drug permeation through the aqueous diffusion layer adjacent to the mucosal surface will also be slow due to their low aqueous solubility. Water-soluble cyclodextrin complexes of these drugs will enhance their diffusion to the mucosal surface leading to enhanced oral bioavailability. Class III drugs are water soluble, but do not easily permeate biological membranes due to, for example, their size and/or extent of hydration. Consequently, formation of hydrophilic drug/cyclodextrin complexes will not enhance their oral bioavailability, but will, if anything, reduce the ability of dissolved drug molecules to partition from the aqueous exterior into the gastrointestinal mucosa. Class IV drugs are water insoluble and do not readily permeate lipophilic biological membranes. These can, for example, be water-insoluble zwitterions or relatively large lipophilic molecules. Hydrophilic water-insoluble compounds such as zwitterions do not readily form cyclodextrin complexes and, thus, hydrophilic cyclodextrins are not likely to improve their oral bioavailability. However, cyclodextrins are able to improve aqueous solubility of some large lipophilic molecules leading to increased drug availability at the mucosal surface. This will frequently lead to increased oral bioavailability.

3.2 Sublingual drug delivery

Sublingual drug delivery is one of the most efficient ways to bypass hepatic first-pass metabolism [78]. However, in order to enter into the systemic circulation the drug must dissolve in the saliva. Due to the small volume of saliva in the mouth, the therapeutic dose has to be relatively small and usually dissolution enhancers must be applied.

In sublingual formulations the complexation of poorly water-soluble drugs with cyclodextrins has been shown to increase the bioavailability of various lipophilic drugs. For example, 2-hydroxypropyl-β-cyclodextrin has been shown to increase the bioavailability of 17β-oestradiol [79,80], androstenediol [81], clomipramine [82] and danazol [83]. In the case of lipophilic compounds, the aqueous solubility and dissolution rate of a drug is usually the rate-limiting step for drug absorption and, after dissolution, the drug readily penetrates through mucosal membranes. Thus, in most studies, the increased bioavailability achieved by cyclodextrins is likely to be due to increased aqueous solubility and drug dissolution rate. However, interactions between cyclodextrins and sublingual mucosa (i.e., cyclodextrins acting as conventional penetration enhancers) cannot be excluded.

From a toxicological point of view the use of cyclodextrins in sublingual formulations is closely related to other forms of oral administration. The large hydrophilic cyclodextrin molecules do not permeate across the sublingual mucosa and, thus, they are eventually swallowed. However, there are some basic differences between sublingual administration and oral administration of cyclodextrin-containing formulations. As discussed earlier, the drug must be released from the inclusion complex before it can be absorbed. This can be a problem for sublingual applications due to the small volume of aqueous saliva and the relatively short residence time. The dissolved drug is removed from the buccal area within few minutes after administration; therefore not allowing enough time for the drug to be released from the cyclodextrin complex.

One limitation in the use of cyclodextrins in sublingual administration is the effect of cyclodextrins on formulation bulk. For example, in the development of sublingual formulations of Δ^9-tetrahydrocannabinol (THC) for the treatment of various medical conditions, the complexation of THC with 2-hydroxypropyl-β-cyclodextrin and randomly methylated β-cyclodextrin was studied. The result showed that the estimated therapeutic dose (1 mg) of THC could form a water-soluble complex with 400 mg of 2-hydroxypropyl-β-cyclodextrin, but formulation bulk of 400 mg is considered too large for sublingual administration. However, the complexation efficiency of randomly methylated β-cyclodextrin with THC was much higher and, thus, the same amount of THC (1 mg) could form a soluble complex with 25 mg of randomly methylated β-cyclodextrin, which made the development of sublingual THC formulations possible [201]. Results from *in vivo* absorption studies showed that sublingual administration of randomly methylated β-cyclodextrin containing THC formulation increases the bioavailability of THC compared with oral administration [84].

3.3 Nasal drug delivery

The nasal route is another effective way to bypass the hepatic first-pass metabolism [85]. Because of the good permeability properties of nasal mucosa, the nasal route has also been studied as a possible administration route for systemic delivery of peptides. However, in order to enter the systemic circulation the drug has to dissolve in the aqueous nasal fluids. In nasal formulations, cyclodextrins are normally used to increase the aqueous solubility of lipophilic drugs. However, lipophilic cyclodextrins can also interact with biological membranes, acting as penetration enhancers, especially in nasal delivery of peptides [75]. Numerous studies have demonstrated that methylated cyclodextrins in particular are efficient absorption enhancers, and this is one reason why they are the most commonly studied cyclodextrins in nasal drug delivery [86]. The first cyclodextrin-based nasal formulations contained steroidal hormones and peptides [76,87-89]. The results were very promising and the most effective cyclodextrins, methylated cyclodextrin derivatives, increased, for example, the bioavailability of progesterone threefold compared with suspension of the same compound [90]. For example, nasal bioavailability of insulin in rats was increased from about 0 to 100% by including methylated cyclodextrins in the formulation [91]. However, much lower insulin bioavailability after nasal administration was later observed in human studies and it is well known that there are large interspecies differences associated with nasal drug delivery [86]. Recently, promising results from nasal delivery of dihydroergotamine [92], midazolam [93], acyclovir [94] and heparins [95] have been reported. Only insignificant amounts of cyclodextrins are absorbed from the nasal cavity. Most cyclodextrins are removed from the cavity by the nasal mucociliary system, which transports cyclodextrins to the oesophagus and ultimately into the gastrointestinal tract. The local toxicity of cyclodextrins after nasal administration is very low. The acute histological effects of the lipophilic methylated cyclodextrins, for example, were close to physiological saline when studied in rats [96]. In addition, the local toxicity of dimethyl-β-cyclodextrin, indicated by ciliary beat frequency, has been shown to be very mild compared with other absorption-enhancing agents and preservatives (e.g., benzalkonium chloride) used in nasal formulations [97].

The amount of cyclodextrins that can be used in nasal formulations is limited by the fact that only 25 – 150 µl of liquid can be sprayed into each nostril [85]. The oestradiol nasal spray Aerodiol® (Servier) represents the successful use of cyclodextrins in nasal applications; each spray delivers 70 µl of solution, which contains 150 µg of oestradiol dissolved in aqueous randomly methylated β-cyclodextrin solution.

3.4 Pulmonary drug delivery

Pulmonary administration of drugs is usually intended for local treatment of diseases (i.e., to treat asthma, chronic obstructive pulmonary disease or other lung diseases) [98]. However, pulmonary drug delivery is also an attractive route for systemic drug delivery. Drug degradation in the gastrointestinal tract and first-pass metabolism can be circumvented by administration via the lungs. Lungs have a large surface area, the blood flow to the lungs is high and the enzymatic activity in the lungs is relatively low, all of which generate good conditions for effective drug absorption. However, pulmonary drug delivery can be limited by low aqueous solubility and slow drug dissolution. Insoluble particles are removed from the lungs by the mucociliary clearance in the upper airways and by macrophages in the alveoli [98]. Cyclodextrins can be of value in pulmonary delivery by increasing the solubility, stability and dissolution rate of water-insoluble and chemically unstable drugs. This can lead to decreased clearance, increased drug absorption and faster onset of drug action. Furthermore, by forming drug/cyclodextrin complexes, a liquid drug can be converted to a solid form, two incompatible drugs can be mixed in a dry powder formulation, bad smells and/or tastes can be reduced, and local drug irritation in the lungs can be reduced.

Cyclodextrins are more readily absorbed from the lungs than from the gastrointestinal tract and this limits the

Table 5. Current marketed drug formulations [154] compared with cyclodextrin formulations (unpublished results and [155]).

	Commercial product	Cyclodextrin formulation
Diazepam i.v. solution (Roche)		
Diazepam	5 mg/ml	5 mg/ml
Propylene glycol	40%	
Ethyl alcohol	10%	
Sodium benzoate/ benzoic acid	5%	
Benzyl alcohol	1.5%	
Water for injection	~ 43%	~ 93%
2-Hydroxypropyl-β-cyclodextrin		6%
Sodium chloride		0.6%
Phenytoin i.v. solution (Parke-Davis)		
Phenytoin sodium	50 mg/ml	50 mg/ml
Propylene glycol	40%	
Alcohol	10%	
Sodium hydroxide	adjust pH to 12	adjust pH to 11
Water for injection	~ 43%	~ 75%
2-Hydroxypropyl-β-cyclodextrin		20%

number of cyclodextrins that can be included in pulmonary formulations but, in general, cyclodextrins that are considered safe for parenteral administration are also considered safe for pulmonary administration [11,99]. Among the cyclodextrins used in pharmaceutical products, γ-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin and sulfobutylether β-cyclodextrin are considered to be the safest for parenteral administration. However, the number of studies dealing with the toxicity or local effects of cyclodextrins on lung cells is very limited. A 72-h treatment with aqueous 0.1 – 1% 2-hydroxypropyl-β-cyclodextrin solutions did not have any significant effect on growth of CRL7272 human lung cells *in vitro* [100]. The toxicity of natural cyclodextrins, 2-hydroxypropyl-β-cyclodextrin and randomly methylated β-cyclodextrin was also studied recently with Calu-3 pulmonary epithelial cell line *in vitro* [101]. The results showed that cyclodextrins are well tolerable in Calu-3 cells and decreased the cell viability only at the high cyclodextrin concentrations.

The number of studies dealing with pulmonary applications of cyclodextrins is also very limited. Studies have been performed using premetered dry powder inhalers, which emit the dose from a pierced blister or capsule [102]. The respirable fraction of salbutamol from Diskhaler® (GlaxoSmithKline) has been increased by complexation with γ-cyclodextrin and dimethyl-β-cyclodextrin [104], and the respirable fraction of beclomethasone dipropionate from Microhaler® has been increased by 2-hydroxypropyl-β-cyclodextrin complexation [104]. Furthermore, the absorption of intratracheally administered drugs has been shown to increase in the presence of various cyclodextrins [105-107]. A recent study with budesonide also showed that cyclodextrin complexes could be used in an inhalation powder without lowering the pulmonary deposition of the drug [108].

3.5 Injectable formulations

Injectable formulations of lipophilic water-insoluble drugs frequently consist of mixtures of water, organic cosolvents and surfactants. Limitations in using organic solvents in injectable formulations include possible drug precipitation, pain, inflammation and haemolysis on injection [109]. Sometimes it is possible to alleviate these side effects by designing a water-soluble prodrug of the lipophilic water-insoluble drug. However, a prodrug will change the pharmacokinetics of the parent drug. For example, due to the gradual metabolism of the prodrug to form the active drug, the onset time of the drug (i.e., the time required for the drug to reach minimum effective plasma concentration) will be delayed [110,111]. Organic solvents and surfactants can be replaced by isotonic aqueous cyclodextrin solutions (**Table 5**). Numerous studies have shown that unlike prodrugs these aqueous cyclodextrin vehicles containing the active drug will in general not alter the intrinsic pharmacokinetics of a drug [10,12]. On parenteral administration, especially after intravenous injection, the drug is both rapidly and quantitatively released from the cyclodextrin complex upon dilution, competitive replacement, and binding of drug molecules to plasma proteins and tissue [113]. However, because cyclodextrins are rapidly eliminated in the urine cyclodextrins can increase renal clearance of lipophilic water-insoluble drugs [112]. Finally, the hydrophilic cyclodextrin derivatives, such as 2-hydroxypropyl-β-cyclodextrin and sulfobutylether β-cyclodextrin, are relatively non-toxic compared with organic solvents and surfactant formulations. Furthermore, as they have a minimal effect on the intrinsic pharmacokinetics of drugs, cyclodextrin-containing formulations are increasingly being used during *in vitro* and *in vivo* screening of new pharmacologically active compounds.

3.6 Ophthalmic drug delivery

In ophthalmology local drug administration in the form of topically applied low viscosity aqueous eye drop solutions is preferred. The outermost layer of the eye cornea is a lipophilic epithelium and, thus, drugs must be somewhat lipophilic to be able to permeate through the cornea into the eye. However, attached to microvilli at the corneal surface is an aqueous layer of about 8 µm thick and, thus, topically applied drugs must be water soluble to be able to penetrate this aqueous diffusion barrier to reach the corneal surface [113]. In addition, only one eye drop, or 0.03 – 0.05 ml, can be applied to the eye, which means that in aqueous eye drop solution the drug dose must be soluble in < 0.05 ml of the aqueous formulation. The average tear volume is only 7 µl and any excess liquid is rapidly spilled onto the skin or drained through the nasolacrimal duct

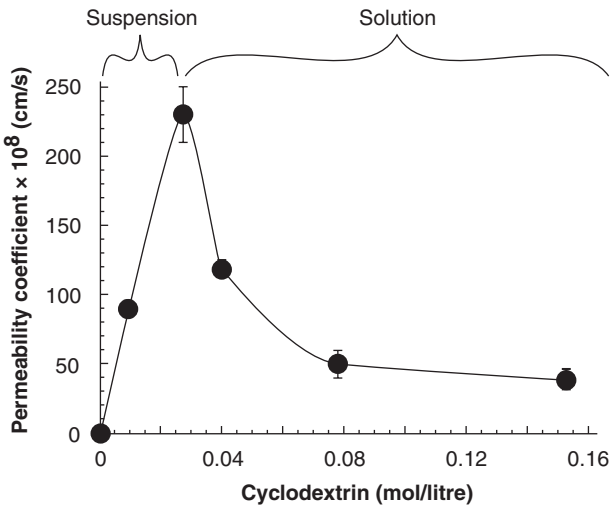


Figure 4. The permeability coefficient of arachidonylethanolamide through the isolated cornea of pigmented rabbits as a function of 2-hydroxypropyl-β-cyclodextrin concentration. The vehicle consisted of 0.5 mg/ml of the drug in suspension or solution containing from 0.000 to 0.155 mol/litre cyclodextrin. Approx. 0.03 mol/litre cyclodextrin was needed to dissolve 0.5 mg/ml of the drug. Modified from reference [156].

into the nose. In addition, continuous secretion of tear fluid limits the contact time of topically applied drugs with the eye surface. Consequently, < 5% of a topically applied drug is absorbed into the eye [114,115]. Through cyclodextrin solubilisation it is possible to increase the dose-to-solubility ratio, making it possible to apply drugs topically that previously could only be given by systemic delivery [115,116]. For example, acetazolamide is a carbonic anhydrase inhibitor that is used to treat glaucoma with oral daily dose as high as 1000 mg. The aqueous solubility of acetazolamide in pure water is 0.7 mg/ml, but in 20% (w/v) aqueous 2-hydroxypropyl-β-cyclodextrin solution it is 7 mg/ml. Addition of water-soluble polymers to the aqueous cyclodextrin solution increases the solubility even further. Thus, it is possible to obtain topically effective acetazolamide eye drop solution through cyclodextrin solubilisation of the drug [117].

Cyclodextrin solubilisation of the drug will increase the amount of dissolved drug at the lipophilic membrane surface (i.e., enhance drug delivery through the aqueous diffusion barrier), but excess cyclodextrin (i.e., more than is needed to dissolve the drug) will decrease the ability of the drug molecules to partition into the lipophilic barrier. Thus, excess cyclodextrin can result in decreased drug delivery through the cornea (**Figure 4**). Cyclodextrins have also been used to reduce ophthalmic drug irritation and to increase chemical stability of drugs in aqueous ophthalmic formulations [115,118].

3.7 Dermal drug delivery

It is generally believed that the main barrier to drug absorption, into and through the skin, is the outermost layer of the skin: stratum corneum. Penetration enhancers used in dermal drug formulations, such as fatty acids and alcohols, penetrate into stratum corneum and temporary decrease its barrier properties. However, only negligible amounts of topically applied hydrophilic cyclodextrins are able to penetrate into the stratum corneum and they have negligible effect on its barrier properties [10,67,68,73]. Numerous studies have shown that excess cyclodextrins do, like in the case of ophthalmic drug delivery (**Figure 4**), decrease drug delivery through excised skin [73]. Cyclodextrins enhance drug delivery through aqueous diffusion layers (i.e., aqueous diffusion barriers), but not through lipophilic barriers such as the stratum corneum. If the drug release is from an aqueous-based vehicle or if an aqueous diffusion layer at the outer surface of the skin is a rate-determining factor in dermal drug delivery, then cyclodextrins can act as penetration enhancers (**Table 3**). However, if drug penetration through the lipophilic stratum corneum is the main rate-determining factor then cyclodextrins are unable to enhance the delivery [74]. It appears that cyclodextrins do enhance hydrocortisone delivery from an unstirred aqueous donor phase through hairless mouse skin, but have no effect on hydrocortisone delivery from a well-stirred donor phase [74,119]. In general, cyclodextrins do not enhance drug delivery from non-aqueous vehicles [68]. For example, it has been shown that both β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin reduce the amount of hydrocortisone released from non-aqueous petrolatum-based vehicles and from w/o cream (water-in-oil emulsion), but enhance the release from both o/w cream (oil-in-water emulsion) and a hydrogel formulation [120]. When applied to excised human skin, cyclodextrins enhanced hydrocortisone delivery from the o/w cream, but reduced the delivery from the non-aqueous petrolatum-based vehicle [121]. As cyclodextrins enhance dermal delivery of drugs by increasing the amount of drug at the surface of the stratum corneum, and conventional penetration enhancers enhance drug delivery by decreasing the barrier function of the stratum corneum, it is possible to obtain an additive effect by combining the two types of enhancers [122,123]. For example, in one study the effects of both 2-hydroxypropyl-β-cyclodextrin and a conventional penetration enhancer (i.e., glycerol monoether extract) on transdermal delivery of testosterone, from o/w cream through hairless mouse skin, was investigated *in vitro* [124]. An ~ 60% increase in the testosterone flux was obtained when cyclodextrin was added to the cream, about a 40% increase occurred when the extract was added to the cream, but about an 80% increase in the flux was observed when both cyclodextrin and the extract were added to the cream.

Cyclodextrins have also been used to reduce permeability of compounds into skin. For example, addition of an excess of 2-hydroxypropyl-β-cyclodextrin to a vehicle containing the UV-absorbing compound oxybenzone (a common sunscreen) (more than needed to solubilising the compound) reduced significantly transdermal permeation of the compound [125]. In addition, studies have indicated that complexation of the

sunscreen enhances its photoprotective effects by preventing permeation of the sunscreen into skin [126].

4. Expert opinion and conclusion

Recent advances in drug development, such as high-throughput screening, have increased the number of drug candidates whose clinical usefulness is hampered by their insolubility in water. Furthermore, the usefulness of a number of drugs and drug candidates is hampered by their chemical or physical instability, or local irritation after administration. Cyclodextrins can alleviate many of these undesirable drug properties. Worldwide there are currently about 30 different cyclodextrin-containing pharmaceutical products on the market in about 14 different types of formulations, including different types of tablets (i.e., conventional, chewing and sublingual tablets), oral capsules, parenteral solutions, suppositories, nasal sprays, eye drop solutions and dermal products. In these products cyclodextrins are used to replace organic solvents in parenteral and topical formulations, to enhance oral bioavailability of Class II and some Class IV drugs, to reduce gastrointestinal irritation and to increase dermal availability of drugs. Furthermore, studies in both humans and animals have shown that cyclodextrins can be used to improve drug delivery from almost any type of drug formulation, but not all. The outcome of a cyclodextrin formulation is highly dependent on the physicochemical properties of the drug being formulated. In addition, the pharmaceutical formulator has to possess a good knowledge of the physicochemical properties of cyclodextrins and their complexes to be able to apply this new technology successfully in drug delivery. Addition of cyclodextrins to existing formulations, without further optimisation, will seldom result in acceptable outcome.

We still lack deeper knowledge of the forces involved in the complex formation. Recent studies have shown that cyclodextrins form both inclusion and non-inclusion complexes, and that those complexes coexist in aqueous solutions. It has also been shown that cyclodextrins form aggregates in aqueous solutions and that those aggregates are able to act as solubilisers in a micellar-like fashion. However, we do not know the exact structures of these non-inclusion complexes and aggregates, nor do we know how they influence drug delivery from cyclodextrin-containing drug formulations. Novel cyclodextrin derivatives for site-specific drug delivery and gene delivery are being synthesised and tested in animals, as well as derivatives in which cyclodextrins are being used as pro-moieties in prodrugs intended for colon drug delivery. In addition, pharmacologically active cyclodextrin derivatives have recently been designed and shown to be clinically effective drugs. Thus, functionality of cyclodextrins will increase rapidly in the coming years.

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Affiliation

Thorsteinn Loftsson PhD[1†], Pekka Jarho PhD[2], Már Másson PhD[1] & Tomi Järvinen PhD[2]
[†]Author for correspondence
[1]University of Iceland, Faculty of Pharmacy, Hagi, Hofsvallagata 53,IS-107, Reykjavik,Iceland
Tel: +354 525 4464; Fax: +354 525 4071;
E-mail: thorstlo@hi.is
[2]University of Kuopio, Department of Pharmaceutical Chemistry, P.O. Box 1627,FIN-70211, Kuopio, Finland

MINIREVIEW

Self-Association of Cyclodextrins and Cyclodextrin Complexes

THORSTEINN LOFTSSON,[1] MÁR MÁSSON,[1] MARCUS E. BREWSTER[2]

[1]Faculty of Pharmacy, University of Iceland, Hofsvallagata 53, IS-107 Reykjavik, Iceland

[2]Johnson&Johnson, Pharmaceutical Research and Developments, Division of Janssen Pharmaceutica N.V., Turnhoutsweg 30, B-2340 Beerse, Belgium

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ABSTRACT: Cyclodextrins are useful functional excipients, which are being used in an ever-increasing way to camouflage undesirable pharmaceutical characteristics, especially poor aqueous solubility. It has generally been assumed that the mechanism whereby cyclodextrins exert their effects, especially their augmentation of solubility, is via the formation of noncovalent, dynamic inclusion complexes. This is a model, which regards drug–cyclodextrin interactions as a discrete phenomenon and ignores the possible interaction of these complexes with one another. It is becoming increasingly apparent that such assumptions may not be universally applicable or all encompassing. Specifically, there is a growing body of evidence that supports the important contribution of non-inclusion-based aspects for drug solubilization by cyclodextrins including surfactant-like effects and molecular aggregation. This short review attempts to assess the available literature for areas in which such non-inclusion mechanisms are apparent and tries to interpret these in the context of a broader working theory as to how cyclodextrins exert their beneficial effects. © 2004 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 93:1091–1099, 2004

Keywords: cyclodextrin; self-association; complexation; non-inclusion; aggregates; phase-solubility; solubilization

INTRODUCTION

It is generally assumed that when drug forms a complex with cyclodextrin, the molecule, in part or in whole, is taken up into the somewhat lipophilic cyclodextrin central cavity. In other words, an inclusion complex of the drug and cyclodextrin is formed.[1] Frequently, the stoichiometry of inclusion complexes is determined by simply fitting the phase-solubility diagrams, that is, the effect of cyclodextrin concentration on total drug solubility, to the appropriate equation without further verification.[2,3] In other instances, the stoichiometry of complexation is verified through Job's plots or nuclear magnetic resonance (NMR) studies, but these are typically performed in dilute solutions in contrast with phase-solubility studies which are based on investigations of saturated drug solutions. Theoretical computer modeling can also be used to assess steric interaction of complexation and have been used to predict the nature of drug–cyclodextrin interaction in either vacuum or ideal solutions. Although these theoretical approaches continue to improve, at present they tend to be highly oversimplified descriptions and ignore important aspects of the formation of cyclodextrin complexes and their structure, especially in concentrated (i.e., nonideal) solutions.

It is well known that both noncyclic oligosaccharides and polysaccharides are able to form non-inclusion complexes.[4–7] It has also been shown that cyclodextrins are, like noncyclic oligosaccharides,

Correspondence to: Thorsteinn Loftsson (Telephone: 354-525-4464; Fax: 354-525-4071; E-mail: thorstlo@hi.is)

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able to form non-inclusion complexes.[8–13] For example, Gabelica et al.[8,9] have reported that α-cyclodextrin forms both inclusion and non-inclusion complexes with α,ω-dicarboxylic acids and that the two types of complexes coexist in aqueous solutions. By comparing α-cyclodextrin complexes with those of maltohexaose, a linear analog of α-cyclodextrin, the authors were able to show that the 1:1 α,ω-dicarboxylic acid/α-cyclodextrin complexes are mainly inclusion complexes whereas 2:1 complexes, formed by additional complex formation between a given acid and a 1:1 complex, are non-inclusion complexes. Even the 1:1 complexes were shown to consist of a mixture of inclusion and non-inclusion complexes. Other investigators have shown that acridine/dimethyl-β-cyclodextrin 2:1 complex is formed when a 1:1 acridine/dimethyl-β-cyclodextrin inclusion complex forms a non-inclusion complex with a second acridine molecule.[13] Furthermore, some 1:2 and 2:2 drug/cyclodextrin complexes have been shown to consist of a mixture of inclusion and non-inclusion complexes.[14] Having said this, in current complexation models, formation of non-inclusion cyclodextrin complexes is generally ignored.

Current stoichiometric models assume that once formed, the hydrated drug/cyclodextrin inclusion complexes are in an ideal solution in which individual complexes are independent of each other. However, several investigators have shown that both cyclodextrins and cyclodextrin complexes can self-associate to form aggregates of two or more cyclodextrin molecules or complexes.[15–26] In some cases, the aggregation results in opalescence of aqueous cyclodextrin solutions[15] but, in most cases, the diameter of the aggregates is much smaller than the wavelength of visible light and, consequently, only clear solutions are observed.[18,19,24,25] Studies have indicated that these water-soluble drug/cyclodextrin complex aggregates are effective solubilizers themselves, solubilizing lipophilic water-soluble drugs through non-inclusion complexation or through formation of micelle-like structures.[23,24] Current stoichiometric models do not account for formation of cyclodextrin aggregates and their effect on drug solubility.

METHODS USED TO DETERMINE STABILITY CONSTANTS OF CYCLODEXTRIN COMPLEXES

In aqueous cyclodextrin solution, free drug molecules are in equilibrium with molecules bound to cyclodextrin molecules. The two most important characteristics of the complexes are their stoichiometry and the numerical values of their stability constants. If m drug molecules (D) associate with n cyclodextrin molecules (CD) to form a complex ($D_m \cdot \mathrm{CD}_n$), the following overall equilibrium is attained:

$$mD + n\mathrm{CD} \overset{K_{m:n}}{\rightleftharpoons} D_m \cdot \mathrm{CD}_n \qquad (1)$$

where $K_{m:n}$ is the stability constant of the drug/cyclodextrin complex. In general, the physicochemical properties of free drug molecules are different from those bound to the cyclodextrin molecules. Likewise, the physicochemical properties of free cyclodextrin molecules are different from those in the complex. In general, any methodology that can be used to observe these changes in additive physicochemical properties may be utilized to determine the stoichiometry of the complexes formed and the numerical values of their stability constants.[3,27] These include changes in solubility, changes in chemical reactivity, changes in UV/VIS absorbance, changes in fluorescence, NMR chemical shifts, changes in drug retention (e.g., in liquid chromatography), changes in pK_a values, potentiometric measurements, and effects on drug permeability through artificial membranes. Furthermore, because complexation will influence the physicochemical properties of the aqueous complexation media, methods that monitor these media changes can be applied to study the complexation. These include measurements of conductivity changes, determinations of freezing-point depression, viscosity measurements, and calorimetric titrations. The stoichiometry of the drug/cyclodextrin complexes and the numerical values of their stability constants should be independent of the methodology applied and sometimes that is the case. Frequently, however, these values will depend on the specific method used for the determination. In Table 1, two methods, a fluorometric procedure and potentiometric approach using ion selective electrode, are compared. The former method utilizes a constant total drug concentration whereas the latter utilizes a constant cyclodextrin concentration. Significant differences were observed, especially when multiple complexes were formed.[28] The stability constants of 1:1 complexes of 5-phenylbarbituric acid and chlorpromazine with β-cyclodextrin, obtained from three different publications, are shown in Table 2. Again, significant discrepancies are observed, even between values obtained from the same publication.

Table 1. Stability Constants of 1-Anilino-8-naphthalenesulfonate (1,8-ANS) and 2(*p*-Toluidinyl)-6-naphthalenesulfonate (2,6-TNS) with β-Cyclodextrin Determined by Fluorescence Method and by Using Ion Selective Electrodes[28,29]

	1,8-ANS	2,6-TNS	
Method	$K_{1:1}$ (M^{-1})	$K_{1:1}$ (M^{-1})	$K_{1:2}$ (M^{-1})
Fluorescence	110 ± 4	1980 ± 84	600 ± 96
Ion selective electrode	86.9 ± 1.2	3737 ± 6	149 ± 2

Itraconazole is a relatively large lipophilic molecule (MW 706). The aqueous solubility of the drug has been estimated to be about 1 ng/mL at neutral pH and about 4 μg/mL at pH 1.[33] Studies have indicated that the drug forms 1:1, 1:2, and 1:3 drug/2-hydroxypropyl-β-cyclodextrin complexes.[33–35] However, significant differences are observed between the determined stability constants (Table 3, p. 1094). An NMR study indicated that the triazole and triazolone rings of the drug molecule could be involved in the 1:2 complex formation.[34] Because of the very low aqueous drug solubility, the NMR study of the itraconazole/cyclodextrin complex was done in pure dimethyl sulfoxide-d_6. The authors argue that because the dielectric constants of dimethyl sulfoxide and water are similar ($\varepsilon = 46.8$ and 80, respectively), the stoichiometry of the complex should be similar. Although the NMR studies, together with the shape of the itraconazole-cyclodextrin phase-solubility profile, indicate that 1:2 itraconazole/2-hydroxypropyl-β-cyclodextrin complex is being formed, the results are inconclusive. In particular, the curvature of the observed A_p-isotherm is such that no linear portion can be defined.

Table 2. Stability Constants ($K_{1:1}$) of 5-Phenylbarbituric Acid (PBA) and Chlorpromazine (CPZ) 1:1 Complexes with β-Cyclodextrin at 25°C

	$K_{1:1}$ (M^{-1}) and Reference	
Method	PBA	CPZ
pH-metric curve fitting	1590[30]	3260[30]
Ultraviolet spectrophotometry	1650[31]	12,000[32]
Circular dichroism	2940[31]	7900[32]
High-performance liquid chromatography	1860[31]	8310[32]
Solubility	3300[31]	—

The stability constants of 1:1 complexes for the un-ionized (the acidic) and the ionized (the basic) forms of ibuprofen and naproxen with 2-hydroxypropyl-β-cyclodextrin are listed in Table 4 (p. 1094). As expected, the complexes of the un-ionized forms appear to have larger stability constants than the ionized, more hydrophilic, forms, but significant variation is observed which is difficult to explain. For example, the stability constant for ionized ibuprofen 1:1 complex with 2-hydoxypropyl-β-cyclodextrin is 1550 M^{-1} when determined by capillary electrophoresis, 533 M^{-1} when determined by a fluorescence method, and only 50 M^{-1} when determined by the phase-solubility method. Likewise, the stability constant for ionized naproxen 1:1 complex with the same cyclodextrin is 540 M^{-1} when determined by a fluorescence method but it has a much lower value when determined by the phase-solubility method. The various values of the stability constants of diflunisal/cyclodextrin 1:1 complexes are shown in Table 5 (p. 1095).

This variation in the observed stability constants could be due to differences in experimental technique, including the concentration of dissolved drug, but under ideal conditions, the different methods should give identical results. Ionic strength can sometimes have variable effect on phase-solubility diagrams. However, it has been shown that ionic strength has negligible effect on the binding of neutral molecules and, most frequently, negligible or negative effect on the binding of ionizable molecules.[24,48,49]

PHASE-SOLUBILITY DIAGRAMS

The stoichiometry of drug/cyclodextrin complexes and the numerical values of their stability constants are frequently obtained from phase-solubility diagrams, that is, plots of drug solubility versus cyclodextrin concentration.[2,50] The phase-solubility technique was developed by Higuchi[2,51] and it is based on research related to how complexes of different complexing agents such as caffeine, polyvinylpyrrolidone, and some aromatic acids affect the aqueous solubility of drugs. These reports refer to π-complexation, but the principle should also apply to monomolecular inclusion complexation. Linear phase-solubility diagrams (Higuchi's A_L-type) indicate that the complex is first order with respect to the complexing agent [$n = 1$ in eq. (1)] and first or higher order with respect to the drug ($m \geq 1$). In

Table 3. Stability Constants of Itraconazole/2-Hydroxypropyl-β-cyclodextrin in Aqueous Solution at pH 2.0 and 25°C

Method and Reference	Solvent	$K_{1:1}$ (M^{-1})	$K_{1:2}$ (M^{-1})
Solubility[35]	Water	3350	90
Solubility[33]	Water	8930	27
Solubility[34]	10% (v/v) propylene glycol in water	120	240
Ultraviolet spectrophotometry[34]	10% (v/v) propylene glycol in water	245	10

this case, the apparent drug solubility (S_{tot}) will be given by:

$$S_{tot} = S_0 + m[D_m \cdot CD] \quad (2)$$

where S_0 is the inherent solubility of the drug in the aqueous complexation medium. If one drug molecule forms a water-soluble complex with one cyclodextrin molecule (i.e., 1:1 complex), then the slope of the linear phase-solubility diagram will be determined by the equation:

$$\text{Slope} = \frac{S_0 K_{1:1}}{S_0 K_{1:1} + 1} \quad (3)$$

where $K_{1:1}$ is the stability constant for the complex. In this case, the slope is always less than unity. If a 2:1 drug/cyclodextrin complex is formed, then the slope of the linear phase-solubility diagram will be determined by the equation:

$$\text{Slope} = \frac{2S_0^2 K_{2:1}}{S_0^2 K_{2:1} + 1} \quad (4)$$

where $K_{2:1}$ is the stability constant of the complex. In this case, the slope of the linear phase-solubility diagram is always less than two.

Positive deviation from linearity (A_P-type phase-solubility diagrams) suggests formation of a higher-order complex with respect to cyclodextrin. The stoichiometry of the system can be probed by curve fitting with a quadratic model. A good fit to this model could suggest formation of a 1:2 drug/cyclodextrin complex:

$$S_{tot} = S_0 + K_{1:1} S_0 [CD] + K_{1:1} K_{1:2} S_0 [CD]^2 \quad (5)$$

where [CD] represents the concentration of free cyclodextrin. A third-order model is suggestive of a 1:3 complex, etc.[3] Here, consecutive complexation is assumed where, for example, a 1:2 complex is formed when one additional cyclodextrin molecule forms a complex with an existing 1:1 complex. Again, it is important to remember that this technique does not indicate whether a given drug forms inclusion complex with cyclodextrin, but only how the cyclodextrin influences the drug

Table 4. Stability Constants of Several Nonsteroidal Antiinflammatory Drugs, Carboxylic Acids, Assuming 1:1 Complexes with 2-Hydroxypropyl-β-cyclodextrin Determined in Aqueous Cyclodextrin Solutions

Drug	Form	pH	Temperature (°C)	Method	$K_{1:1}$ (M^{-1})	Reference
Ibuprofen	Un-ionized	4.6	30	Solubility	1740	[36]
(pK_a 5.2)		6[a]	25	Solubility	5400	[37]
	Ionized	7	20	Fluorescence	533	[38]
	Ionized	7.4	25	Capillary electrophoresis[b]	1550	[39]
	Ionized	7.5	30	Solubility	50	[36]
Naproxen	Un-ionized	[c]	25	Solubility	1670	[40]
(pK_a 4.2)	Un-ionized	~1	35	Fluorescence	2600	[41]
	—	4.6	30	Solubility	1400	[36]
	Mainly ionized	5[a]	25	Solubility	2580	[42]
	Mainly ionized	6[a]	25	Solubility	2600	[37]
	Ionized	7.4	30	Solubility	21	[36]
	Ionized	[c]	25	Solubility	331	[40]
	Ionized	~9	35	Fluorescence	540	[41]

[a] Unbuffered solution.
[b] The constant was determined by Scatchard analysis.
[c] Calculated values based on measurements in a series of aqueous buffer solutions.

Table 5. Stability Constant of Sodium Diflunisal/Cyclodextrin 1:1 Complexes in Aqueous Solutions

Method	Cyclodextrin	Temperature (°C)	$K_{1:1}$ (M^{-1})	Reference
UV spectrophotometry	HPβCD	25	5070	43
^{19}F-NMR	HPβCD	25	2030[a]	43
Dialysis	HPβCD	24	3890	44
Microcalorimeter	HPβCD	25	3390	44
Potentiometric	HPβCD	25	5570	45,46
UV spectrophotometry	βCD	25	181,000	47
Potentiometric	βCD	25	78,300	46
UV spectrophotometry	γCD	25	55,000	47
Potentiometric	γCD	25	88,200	46

The pK_a of diflunisal is 3.0.
[a]For the C2′ signal.

solubility. Phase-solubility studies are performed in aqueous solutions saturated with the drug where formation of higher-order complex aggregates is more likely than in diluted unsaturated solutions.

Although correlation is often found between phase-solubility diagrams and the stoichiometry of drug/cyclodextrin complexes determined by other means such as NMR, some discrepancies can be found in the literature. For example, the slopes of linear (i.e., A_L-type) phase-solubility diagrams of the sodium salts of ibuprofen and diflunisal in aqueous pH 6.0 phosphate buffer solution containing 2-hydroxypropyl-β-cyclodextrin are 1.2 and 1.3, respectively.[24] This indicates that the complexes formed are first order with respect to cyclodextrin but second or higher order with respect to the sodium salts of ibuprofen and diflunisal. However, all other studies (including NMR investigations, Job's plots, and molecular modeling) indicate that the complexes formed are first order with respect to both cyclodextrin and the drugs. In addition, it has been shown that both diflunisal/cyclodextrin and ibuprofen/cyclodextrin complexes possess solubilizing properties in and of themselves, most likely by solubilizing drugs through non-inclusion solubilization or solubilization by complex aggregates.[23,24]

Because of electrostatic repulsions between the individual cyclodextrin molecules, sodium sulfobutylether β-cyclodextrin are thought to form exclusively 1:1 drug/cyclodextrin complexes.[52] However, the phase-solubility diagrams of sulfobutylether β-cyclodextrin are frequently of A_P-type. For example, the phase-solubility diagrams of cholesterol[24] and some pilocarpine prodrugs[53] in aqueous sulfobutylether β-cyclodextrin solutions are of A_P-type, indicating formation of higher-order complexes with regard to cyclodextrin, but the profiles could not be fitted to the appropriate equations. It has been pointed out that A_P-type profiles have a strong resemblance to phase-solubility diagrams of lipophilic water-insoluble compounds in aqueous surfactant solutions. Thus, it has been suggested that 1:1 drug/cyclodextrin inclusion complexes form water-soluble non-inclusion complexes with additional drug molecules to give rise to A_P-type phase-solubility diagrams.[24] In fact, it has been shown that the solubility of cyclosporin A is about 33% higher in aqueous 2-hydroxypropyl-β-cyclodextrin solutions that had previously been saturated with cholesterol compared with solutions that had not been saturated with cholesterol.[54] It is possible that the A_P-type phase-solubility diagrams observed for itraconazole in aqueous 2-hydroxypropyl-β-cyclodextrin solutions may not be due to formation of higher-order complexes with respect to cyclodextrin but rather due to additional solubilization of itraconazole by the 1:1 complexes.

CYCLODEXTRINS AND DRUG PERMEABILITY

Cyclodextrins are relatively large (MW from almost 1000 to more than 2000) hydrophilic molecules and thus cyclodextrins and their complexes do not efficiently permeate lipophilic biological membranes. Hydrophilic cyclodextrins enhance drug delivery through aqueous diffusion barriers but have no effect on drug permeation

through lipophilic membrane barriers.[55,56] The ability of cyclodextrins and their complexes to permeate semipermeable cellophane membranes depends on the molecular weight cutoff (MWCO) of the membranes.[25,56] Only relatively small drug molecules are able to permeate membranes with a MWCO of 500 but cyclodextrins and their complexes are able to permeate membranes with MWCO > 3000. Thus, it is possible to use semipermeable cellophane membranes to determine the stability constants of drug/cyclodextrin complexes.[57] The phase solubility of hydrocortisone in aqueous 2-hydroxypropyl-β-cyclodextrin solutions is of A_L-type, indicating that a 1:1 drug/cyclodextrin complex is being formed and the stability constant of the 1:1 complex has been determined to be 1400 M^{-1}.[25] The flux of hydrocortisone was determined from aqueous cyclodextrin solutions saturated with the drug through a series of membranes with MWCO from 500 to 14,000 (Fig. 1). Based on Fick's first law of passive diffusion, there should be a linear relationship between the hydrocortisone flux through the MWCO 500 membrane and the unbound hydrocortisone concentration in the donor solution, but in the case of the membranes with a MWCO of $\geq$6000, the linear relationship should be between the total hydrocortisone concentration (i.e., bound and unbound to cyclodextrin) and the flux. The concentration of unbound hydrocortisone in the hydrocortisone-saturated cyclodextrin solutions is constant and equal to the inherent solubility of the drug. Thus, in accordance to Fick's first law,

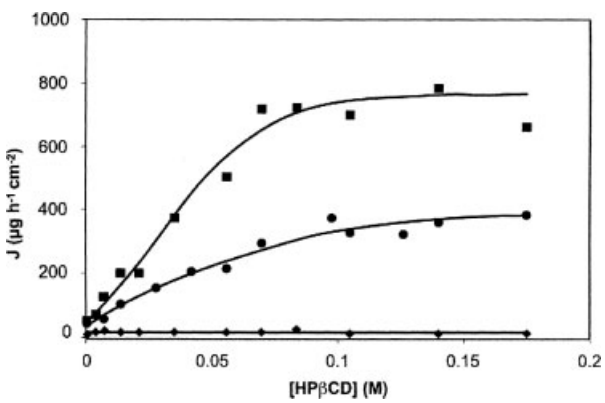

Figure 1. The effect of 2-hydroxypropyl-β-cyclodextrin (HPβCD) concentration on the flux (J) of hydrocortisone from aqueous solutions, saturated with hydrocortisone, through a semipermeable cellophane membrane; MWCO 500 (◆), MWCO 6000–8000 (●), MWCO 12,000–14,000 (■). From Ref.[25] with permission.

no increase in the flux through the MWCO 500 membrane was observed as the total concentration of hydrocortisone in the donor phase was increased (Fig. 1). However, the same solutions should have displayed a linear relationship when measured through the other two membranes with MWCO greater than the molecular weight of the hydrocortisone/cyclodextrin 1:1 complex. But the profiles show a strong negative deviation from linearity (Fig. 1). This deviation from linearity is thought to be due to self-association of cyclodextrin and cyclodextrin complexes to form aggregates that are unable to permeate the semipermeable membranes.[22,24] At increased concentrations, the hydrocortisone, cyclodextrin, and their complexes must form aggregates that have total molecular weight >6000–14,000. Thus, the actual stoichiometry of the hydrocortisone/cyclodextrin complex is much more complex that what can be anticipated from the simple hydrocortisone/cyclodextrin A_L-type phase-solubility diagram.

CONCLUSIONS

In the classical cyclodextrin chemistry, it is assumed that when a drug molecule forms a complex with cyclodextrin, then some given lipophilic moiety of the drug molecule enters into the hydrophobic cyclodextrin cavity. In other words, that an inclusion complex of the cyclodextrin and drug is always formed. It is also assumed that once formed, the hydrated drug/cyclodextrin complexes are in an ideal solution in which individual complexes are independent of each other. Studies have shown that this is a significant oversimplification of a much more involved system. Cyclodextrins are able to form both inclusion and non-inclusion complexes. In addition, cyclodextrins and their complexes form water-soluble aggregates in aqueous solutions and these aggregates are able to solubilize lipophilic water-insoluble drugs through non-inclusion complexation or micelle-like structures.

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