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Cyclodextrins as pharmaceutical solubilizers ☆

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Abstract

Cyclodextrins are useful functional excipients that have enjoyed widespread attention and use. The basis for this popularity from a pharmaceutical standpoint, is the ability of these materials to interact with poorly water-soluble drugs and drug candidates resulting in an increase in their apparent water solubility. The mechanism for this solubilization is rooted in the ability of cyclodextrin to form non-covalent dynamic inclusion complexes in solution. Other solubilizing attribute may include the ability to form non-inclusion based complexes, the formation of aggregates and related domains and the ability of cyclodextrins to form and stabilize supersaturated drug solutions. The increase in solubility also can increase dissolution rate and thus improve the oral bioavailability of BCS Class II and IV materials. A number of cyclodextrin-based products have reached the market based on their ability to camouflage undesirable physicochemical properties. This review is intended to give a general background to the use of cyclodextrin as solubilizers as well as highlight kinetic and thermodynamic tools and parameters useful in the study of drug solubilization by cyclodextrins.
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Keywords: Cyclodextrin; Toxicology; Regulatory status; Solubility; Stability constants; Phase–solubility; Supersaturation; Additives

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List of selected symbols

K	equilibrium constant
K_c	complexation or stability constant (equilibrium constant for a drug-cyclodextrin interaction)
K_d	dissociation constant (i.e., $1/K_c$)
$K_{n:m}$	complexation constant associated with the interaction of *n* drug molecules with *m* cyclodextrin molecules
$K_{1:1}$	complexation constant for the interaction of one drug molecule with one cyclodextrin molecule
D_o	intrinsic drug solubility
D_t	total drug in solution (complexed and uncomplexed)
CD_t	total cyclodextrin in solution (complexed and uncomplexed)
k_o	first order rate constant
k_c	first order rate constant for a drug included into a cyclodextrin molecule
k_{on}	first order rate constant for the association of a drug with a cyclodextrin cavity
k_{off}	first order rate constant for the dissociation of a drug from a cyclodextrin cavity

1. Introduction

In 1891 a French scientist, A. Villiers, described a bacterial digest that he had isolated from starch [1,2]. Experimental results indicated that the substance was a dextrin and Villiers named it “cellulosine”. Later an Austrian microbiologist, Franz Schardinger, described two crystalline compounds α-dextrin and β-dextrin which he had isolated from a bacterial digest of potato starch. Schardinger identified β-dextrin as Villiers' “cellulosine” [3,4]. Now these compounds are commonly called cyclodextrins (i.e. α-cyclodextrin (αCD) and β-cyclodextrin (βCD)) or less commonly cyclomaltodextrins (i.e. cyclomaltohexaose and cyclomaltoheptaose) or cycloamyloses (i.e. cyclohexaamylose and cycloheptaamylose). γ-Cyclodextrin (γCD; cyclomaltooctaose or cyclooctaamylose) was first described in 1935 by Freudenberg and Jacobi [5]. In the years following these discoveries, large ring cyclodextrins (LR-CDs) were discovered [6,7]. Presently only αCD, βCD and γCD, as well as some of their derivatives have advanced to the market. From 1935 to 1955 Freudenberg, Cramer and their co-workers identified the chemical structure of CDs, their general physicochemical properties and their abilities to form complexes [8,9]. During this period, only small amounts of relatively impure CD could be produced which hampered industrial exploitation of these novel oligosaccharides. The biotechnological advances that occurred in the 1970's lead to dramatic improvements in production of highly pure αCD, βCD and γCD, transforming them from expensive chemical oddities to affordable industrial excipients [10]. The interest in CDs as judged by the number of publications and patents has expanded exponentially as illustrated in Fig. 1. Of the 39,679 total publications (as of March 2007), a significant portion are dedicated to the use of the material in pharmaceutics as a solubilizer. For the two most important pharmaceutically relevant CD derivatives, hydroxypropyl-β-cyclodextrin (HPβCD) and sulfobutylether-β-cyclodextrin (SBEβCD), publications from the 2000's reflect the growing interest in these excipients (Fig. 2) as well as their use in novel pharmaceutical applications (Fig. 3) [11].

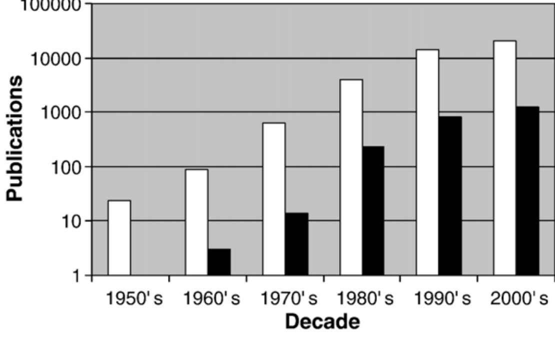


Fig. 1. Log plot of the of publications related to cyclodextrins (white bars) and to pharmaceutical application involving solubilization (black bars) by cyclodextrins, as indicated by SciFinder (ACS, Columbus, OH) in March, 2007.

The world's first CD-containing pharmaceutical product, prostaglandin E2/βCD (Prostarmon E™ sublingual tablets), was marketed in Japan in 1976. Twelve years later, the first European CD-based pharmaceutical product, piroxicam/βCD (Brexin® tablets), was marketed and in 1997, the first US-approved product, itraconazole/2-hydroxypropyl-βCD oral solution (Sporanox®) was introduced [11,12]. Worldwide, 35 different drugs are currently marketed as solid or solution-based CD complex formulations (Table 1) [13,14]. In these pharmaceutical products, CDs are mainly used as complexing agents to increase the aqueous solubility of poorly water-soluble drugs, to increase their bioavailability and stability [15–17]. In addition, CDs can be used to reduce or prevent gastrointestinal and ocular irritation, reduce or eliminate unpleasant smells or tastes [18,19], prevent drug–drug or drug–additive interactions, as well as to convert oils and liquid drugs into microcrystalline or amorphous powders [9]. The goal of this review of the physicochemical properties of CDs is to highlight the utility of these materials as solubilizers, with emphasis given to their pharmaceutical applications.

1.1. The chemical structure of CDs and their production

CDs are cyclic oligosaccharides derived from starch containing six (αCD), seven (βCD), eight (γCD), nine (δCD), ten (εCD) or more (α-1,4)-linked α-D-glucopyranose units (Table 2) [6,7,20]. Due to the chair conformation of the glucopyranose units, the CDs take the shape of a truncated cone or torus rather than a perfect cylinder (Table 2). The hydroxyl functions are

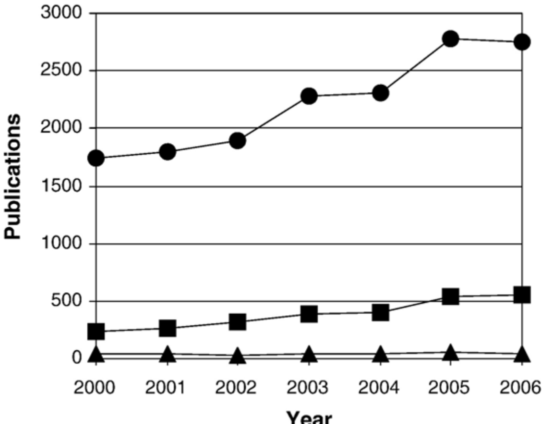


Fig. 2. Evolution in cyclodextrin publications in the 2000's (●All Cyclodextrins, ■-HPβCD and ▲-SBEβCD) (SciFinder, Columbus, OH).

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 of the CD molecule is lined with skeletal carbons and ethereal oxygens of the glucose residue, which gives it a relatively lipophilic character [20–25]. The polarity of the cavity has been estimated to be similar to that of an aqueous ethanolic solution [25]. In aqueous solutions, the hydroxy groups form hydrogen bonds with the surrounding water molecules resulting in a hydration shell around the dissolved CD molecule [26–29].

Schardinger had shown that *Bacillus macerans* is the microbe responsible for the formation of CDs. Treatment of starches with amylase from *B. macerans* gives a crude mixture of αCD (~60%), βCD (~20%) and γCD (~20%) together with small amounts of CDs with more that 8 glucopyranose units [30]. The mixture was difficult to purify and frequently contained several other linear and branched dextrins together with small amounts of proteins and other materials. The use of genetic engineering has made possible the isolation of different types of cyclodextrin glucosyl transferases (CGTases) that are both more active and more specific towards production of predominantly αCD, βCD or γCD than previous production methodologies. The enzymes recognize the 6, 7 or 8 glucopyranose units from the non-reducing terminus of an amylose, the linear component of starch, and alters the adjacent α-1,4-linkage, transferring it to the C-4 position to produce αCD, βCD or γCD [31,32]. Genetically engineered CGTases together with other technological and process innovations has made highly purified αCD, βCD and γCD available as pharmaceutical excipients [33]. In 1970, βCD was only available as a fine chemical at a price of about 2000 USD per kg. Today the annual βCD production is close to 10,000 tons and the commodity price is now about 5 USD per kg. The LR-CDs containing from 9 to 35 glucopyranose units have been purified and characterized [6,7] but LR-CDs are, in general, not good as solubilizers for most drugs (MW < 1000 Da) as are αCD, βCD and γCD. Furthermore, it is still difficult to produce purified LR-CDs in aqueous solutions since LR-CDs are less chemically stable than αCD, βCD or γCD. Consequently, LR-CDs have found little application as solubilizers within pharmaceutical development.

1.2. Chemically modified CDs

The natural CDs, in particular βCD, are of limited aqueous solubility meaning that complexes resulting from interaction of lipophiles with these CDs may also be poorly soluble resulting in precipitation of the solid CD complexes from water and other aqueous systems. In fact, the aqueous solubility of the natural CDs is much lower than that of the comparable acyclic dextrins. This is thought to be due to relatively strong intramolecular hydrogen bonding in the crystal lattice. Substitution of any of the hydrogen bond-forming hydroxyl groups, even by lipophilic functions, results in dramatic improvement in their aqueous solubility [23]. CD derivatives of pharmaceutical interest (Table 3) include the hydroxypropyl derivatives of βCD and γCD (i.e. HPβCD and HPγCD), the randomly methylated βCD (RMβCD), sulfobutylether βCD (SBEβCD), and the so-called branched CDs such as maltosyl-βCD (G_2βCD) [20–22,34–36].

1.3. Toxicological considerations

CDs are associated with MW ranging from almost 1000 to over 2000 Da and are hydrophilic with a significant number of H-donors and acceptors and, thus, are not significantly absorbed from the gastrointestinal tract in their intact form. The natural αCD and βCD, unlike γCD, cannot be hydrolyzed by human salivary and pancreatic amylases [35,37]. However, both αCD and βCD can be fermented by the intestinal microflora. αCD can be found in one marketed parenteral solution and in tablet formulations. Oral administration of αCD is, in general, well tolerated and is not associated with significant adverse effects [38,39]. Only small fractions of αCD are absorbed intact from the gastrointestinal tract and it is mainly excreted unchanged in the urine after iv injection (Table 4). βCD can be found in numerous marketed oral dosage forms as well as in topical, buccal and rectal drug formulations. βCD can not be given parenterally due to its low aqueous solubility and adverse effects (e.g. nephrotoxicity) but it is essentially non-toxic when given orally. After oral administration, the non-toxic effect level of βCD was determined to be 0.7–0.8 g/kg/day in rats and about 2 g/kg/day in dogs [40]. βCD is a common food

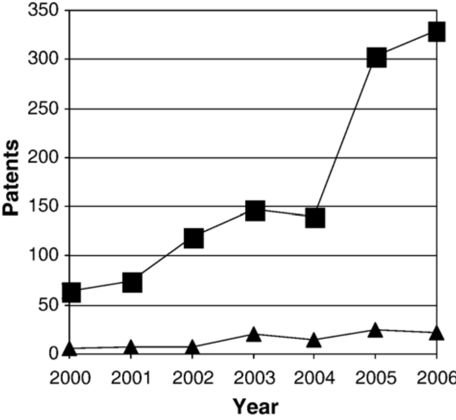


Fig. 3. Patents issued associated with HPβCD(■) and SBEβCD (▲) use in the 2000's (SciFinder, Columbus, OH).

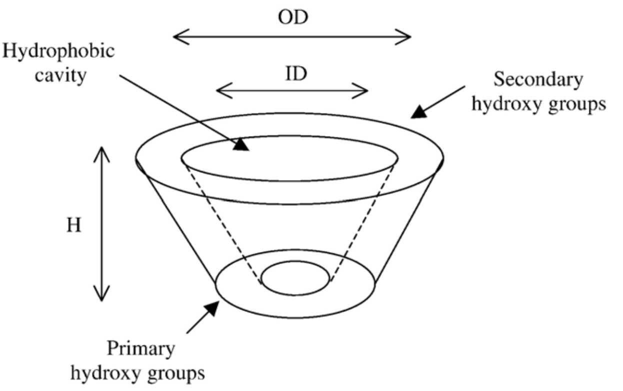
Table 1
Some marketed pharmaceutical products containing cyclodextrins

Drug/cyclodextrin	Trade name	Formulation	Company (country)
α-Cyclodextrin (αCD)			
Alprostadil	Caverject Dual	i.v. solution	Pfizer (Europe)
Cefotiam-hexetil HCl	Pansporin T	Tablet	Takeda (Japan)
OP-1206	Opalmon	Tablet	Ono (Japan)
PGE_1	Prostavastin	Parenteral solutions	Ono (Japan); Schwarz (Europe)
β-Cyclodextrin (βCD)			
Benexate HCl	Ulgut, Lonmiel	Capsule	Teikoku (Japan); Shionogi (Japan)
Cephalosporin	Meiact	Tablet	Meiji Seika (Japan)
Cetirzine	Cetrizin	Chewing tablet	Losan Pharma (Germany)
Chlordiazepoxide	Transillium	Tablet	Gador (Argentina)
Dexamethasone	Glymesason	Ointment, tablet	Fujinaga (Japan)
Dextromethorphan	Rynathisol		Synthelabo (Europe)
Diphenhydramin and chlortheophyllin	Stada-Travel	Chewing tablet	Stada (Europe)
Iodine	Mena-Gargle	Solution	Kyushin (Japan)
Meloxicam	Mobitil	Tablet and suppository	Medical Union Pharmaceuticals (Egypt)
Nicotine	Nicorette	Sublingual tablets	Pfizer (Europe)
Nimesulide	Nimedex	Tablets	Novartis (Europe)
Nitroglycerin	Nitropen	Sublingual tablet	Nihon Kayaku (Japan)
Omeprazole	Omebeta	Tablet	Betafarm (Europe)
PGE_2	Prostarmon E	Sublingual tablet	Ono (Japan)
Piroxicam	Brexin, Flogene, Cicladon	Tablet, suppository	Chiesi (Europe); Aché (Brazil)
Tiaprofenic acid	Surgamyl	Tablet	Roussel-Maestrelli (Europe)
2-Hydroxypropyl-β-cyclodextrin (HPβCD)			
Alfaxalone			
Cisapride	Propulsid	Suppository	Janssen (Europe)
Hydrocortisone	Dexocort	Solution	Actavis (Europe)
Indomethacin	Indocid	Eye drop solution	Chauvin (Europe)
Itraconazole	Sporanox	Oral and i.v. olutions	Janssen (Europe, USA)
Mitomycin	MitoExtra, Mitozytrex	i.v. infusion	Novartis (Europe)
Sulfobutylether β-cyclodextrin sodium salt (SBEβCD)			
Aripiprazole	Abilify	im solution	Bristol-Myers Squibb (USA); Otsuka Pharm. (USA)
Maropitant	Cerenia	Parenteral solution	Pfizer Animal Health (USA)
Voriconazole	Vfend	i.v. solution	Pfizer (USA, Europe, Japan)
Ziprasidone mesylate	Geodon, Zeldox	im solution	Pfizer (USA, Europe)
Randomly methylated β-cyclodextrin (RMβCD)			
17β-Estradiol	Aerodiol	Nasal Spray	Servier (Europe)
Cloramphenicol	Clorocil	Eye drop solution	Oftalder (Europe)
Insulin		Nasal spray	Spain
2-Hydroxypropyl-γ-cyclodextrin (HPγCD)			
Diclofenac sodium salt	Voltaren	Eye drop solution	Novartis (Europe)
Tc-99 Teoboroxime	CardioTec	i.v. solution	Bracco (USA)

additive. The metabolism of γCD closely resembles that of starch and linear dextrins [41]. Only very small amounts of γCD are absorbed intact from the gastrointestinal tract and it is mainly excreted unchanged in the urine after iv injection. Oral administration of 8 g γCD or 8 g maltodextrin to humans did not reveal any differences in gastrointestinal tolerance of these two oligosaccharides [42].

Hydrophilic CDs, namely HPβCD and SBEβCD, are considered non-toxic at low to moderate oral and intravenous doses [35,36]. HPβCD is much more water-soluble and more toxicologically benign than the natural βCD [43,44]. It can be found in several marketed drug formulations (Table 1) with oral dosing of up to 8 g HPβCD/day and intravenous dosing of up to 16 g HPβCD/day (Itraconazole®) [43,44]. HPβCD has been shown to be well tolerated in humans, with the main adverse event being increased incidents of soft stools and diarrhea (at dosage of 16–24 g HPβCD/day for 14 days) [35,43,44]. The biological half-life of HPβCD in humans after intravenous injection is about 1.7 h and its apparent volume of distribution (V_d) is 0.2 l/kg. After intravenous injection, HPβCD is almost exclusively eliminated through the kidneys via glomerular filtration. As such, administration of the excipient to patients with severe renal insufficiency (creatinine clearance < 30 min^{-1}) should be avoided. The oral bioavailability of HPβCD in humans is between 0.5 to 3.3% with 50 to 65% of the oral

Table 2
Characteristics of the natural cyclodextrins αCD, βCD and γCD



Cyclodextrin	Number of glucose units	Dimensions (nm) H	OD	ID	$t_{1/2}$ of ring opening (h)	Mean $K_{1:1}$ [a] (M^{-1})
α-Cyclodextrin (αCD)	6	0.78	1.37	0.57	33	130 ± 8
β-Cyclodextrin (βCD)	7	0.78	1.53	0.78	29	490 ± 8
γ-Cyclodextrin (γCD)	8	0.78	1.69	0.95	15	350 ± 9

[a] Stability constants (binding constants) of 1:1 guest/CD complexes in aqueous solutions at 25 ± 5 °C [59]. Population mean ± standard deviation.

Table 3
Structural and physicochemical properties of selected cyclodextrin of pharmaceutical interest

Cyclodextrin	*n*	*R*=H or	Substance [a]	MW [b] (Da)	Solubility in water [c] (mg/ml)	Indicative bulk price ($US/kg) [d]
α-Cyclodextrin (αCD)	0	‾H	0	972	145	45
β-Cyclodextrin (βCD)	1	‾H	0	1135	18.5	5
2-Hydroxypropyl-β-cyclodextrin (HPβCD; Kleptose® HPB)	1	‾$CH_2CHOHCH_3$	0.65	1400	>600	300
Sulfobutylether β-cyclodextrin sodium salt (SBEβCD; Captisol®)	1	‾$(CH_2)_4SO_3^-$ Na^+	0.9	2163	>500	–
Randomly methylated β-cyclodextrin (RMβCD)	1	‾CH_3	1.8	1312	>500	350
6-O-Maltosyl-β-cyclodextrin ($G_2\beta$CD)	1	Maltosyl‾	0	1459	>1500	–
γ-Cyclodextrin (γCD)	2	‾H	0	1297	232	80
2-Hydroxypropyl-γ-cyclodextrin (HPγCD)	2	‾$CH_2CHOHCH_3$	0.6	1576	>500	400

[a] Average number of substituents per glucose repeat unit.
[b] MW: Molecular weight.
[c] Solubility in pure water at approx. 25 °C. Some values from refs. [25,216,217].
[d] Approximate bulk price given as the price of 1 kg in US dollars. The price will depend on purity and technological grade of the CD.

dose excreted intact in the feces with the remainder mainly being metabolized by the intestinal microflora. Fewer published references on the toxicological potential of SBEβCD are available however this CD derivative can be found in several marketed product, including voriconazole parenteral solution (Vfend®). Voriconazole (10 mg/mL) is solubilized in a solution containing 16%w/v of SBEβCD. At a daily drug dosage of 6 to 12 mg drug/kg BW, a SBEβCD dose of between 6 and 14 g are expected [45]. As with Sporanox®, the i.v. solution is diluted two-fold with saline prior to drug dosing. The available toxicological information on $G_2\beta$CD and HPγCD is even more limited. $G_2\beta$CD is not yet available in a marketed product but

Table 4
Safety overview of selected cyclodextrins

Cyclodextrin	The pharmacokinetics in rats [a]			Acute toxicity, LD_{50} rat (g/kg) [b]		Maximum dosage in marketed products (mg/day)	
	$t_{1/2}$ after iv injection (min)	Fraction excreted unchanged in urine	Oral absorption	IV	Oral	IV	Oral
αCD	25	~90%	2–3%	0.5-0.8	>10	1.3	
βCD	20	~90%	1–2%	1	19	Not for parenteral usage	170
HPβCD	20	~90%	≤3%	10	>2	16,000	8000
SBEβCD				>15	>10	6000–14,000	–
RMβCD	18	>95%	0.5–12%	1.5–2.1	>8	Not for parenteral usage	–
$G_2\beta$CD	23				>5	No product	No product
γCD	20	90%	<0.02%	4	>8	No product	No product
HPγCD					>2		

[a] From [14,35,48,218–220].
[b] From [38,39,221].

HPγCD is found in two products, i.e. an eye drop solution formulation and a parenteral diagnostic product (Table 1).

Lipophilic CD derivatives, such as the methylated CDs, are absorbed to a somewhat greater extent from the gastrointestinal tract into the systemic circulation and have been shown to be toxic after parenteral administration [35]. Presently, oral administration of methylated βCD is limited by its potential toxicity. The oral bioavailability of RMβCD is about 5% in rats and with more than 90% of the material excreted unchanged with feces. More than 95% of RMβCD is excreted unchanged with urine after intravenous injection to rats [46].

The hemolytic effect of CDs on human erythrocytes in phosphate buffered saline are in the order methylated βCDs > βCD > HPβCD ≈ G_2βCD > αCD > γCD > HPγCD > SBEβCD [35,36,47]. There appears to be a correlation between the hemolytic activity and the ability of the CDs to bind or extract cholesterol from the membranes [35]. This *in vitro* cellular lysis study, as well as other comparable *in vitro* studies using intestinal cells, *E. coli* bacterial cells, human skin fibroblasts and liposomes, do not indicate *in vivo* toxicity but rather provide a method to classify CDs according to their potential to destabilize or disrupt cellular membranes [36]. In humans, the acceptable daily oral intakes (ADI) of the natural CDs and RMβCD are 1.4 g for αCD, 0.35 g for βCD, 10 g for γCD and 0.07 g for RMβCD [48].

1.4. Regulatory status

The regulatory status of CDs continues to evolve [13,14]. βCD is listed in a number of pharmacopoeia sources including the US Pharmacopoeia/National Formulary (USP/NF), European Pharmacopoeia (Ph.Eur.) and Japanese Pharmaceutical Codex (JPC). αCD is similarly listed in the Ph.Eur., USP/NF and JPC and γCD is referenced in the JPC and will soon be included in the Ph.Eur. and USP/NF. A monograph for HPβCD is available in the Ph.Eur. and a draft has been circulated for the USP/NF. Other derivatives are not yet compendial but efforts are underway for their inclusion. αCD, βCD and γCD were also introduced into the generally regarded as safe (GRAS) list of the FDA for use as a food additive in 2004, 2001 and 2000, respectively, and HPβCD is cited in the FDA's list of Inactive Pharmaceutical Ingredients. SBEβCD is also available in various dosage forms and is also listed in the FDA's compilation of Inactive Pharmaceutical Ingredients. Consensus seems to be building among regulators that CDs 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.

2. Solubilizing effects of CDs

CDs interact with poorly-water soluble compound to increase their apparent solubility. The mechanisms by which this solubilization occur will be discussed in Section 2.1 but the most prominent of these is inclusion complex formation in which the guest and host molecules are in dynamic equilibrium with the complex [20]. The increased apparent solubility can enable solution-based dosage forms such as parenteral i.v. formulation and oral liquids as illustrated in Table 1. In addition, increasing the apparent solubility of a drug can, through the Noyes–Whitney equation, increase drug dissolution rate and for compounds whose oral bioavailability is limited by solubility or dissolution rate, can act to increase oral bioavailability [49–51]. A useful approach in assessing where CDs can be applied in this context is the Biopharmaceutical Classification System (BCS). The BCS divides drugs and drug candidates into 4 classes based on their solubility and permeability characteristics [52–54]. Soluble, permeable drugs are termed Class I compounds with oral bioavailability only being limited by the rate at which they reach appropriate sites of absorption in the gastrointestinal (GI) tract. Class II drugs are poorly soluble but permeable through the gut meaning that oral adsorption is limited by drug solubility and dissolution rate. Class III compounds are soluble but poorly permeable meaning that oral bioavailability is limited by the barrier properties of the GI tract. Finally, Class IV compounds are both insoluble and poorly permeable combining the limitations of both Class II and III materials. Thus, CD intervention is most applicable to Class II and IV compounds and the use of CDs can alter the properties of these classes such that they become Class I-like in behavior [13,55].

The use of CDs is often preferred to organic solvents from both a toxicological perspective but also from a mechanistic point of view. Based on inclusion complex formation, CDs often solubilize compounds as a linear function of their concentration. This means that as a solution is administered, both the drug and CD concentration are reduced in a linear manner suggesting that precipitation is, at least theoretically, not likely after either oral or i.v. dosing. Organic solvents, on the other hand, solubilize solutes as a log function of their concentration as described by the extended Hildebrand equation [56,57]. This log-linear relationship means that as an organic solvent is introduced into an aqueous environment, the solubilizing power of the formulation is rapidly lost and precipitation can occur. Depending on the nature of this precipitation, the drug can be effectively removed from the equilibria required for absorption from the GI tract. For parenteral liquids, precipitation can occur at the site of injection or at other loci in the body. Once included in the CD cavity, the guest molecule may be released through complex dilution, by replacement of the included guest by some other suitably sized molecule such as dietary lipids or, if the complex is located in close approximation to a lipophilic biological membrane such as the oral mucosa, the guest may be transferred to the matrix for which it has the highest affinity. As reviewed by Stella et al., parenteral administration for the vast majority of CD complexes is thought to be associated with complete and almost instantaneous dissociation via dilution of the complex [58]. In cases where the possibility of dilution is more limited, as in ophthalmic applications, factors associated with partitioning and secondary equilibria may be the main mechanisms for drug release.

2.1. Complexation

The central CD cavity provides a lipophilic microenvironment into which suitably sized drug molecules may enter and

include. No covalent bonds are formed or broken during the drug/CD complex formation and in aqueous solutions, the complexes are readily dissociated. The rates for formation and dissociation of drug/CD complexes are very close to the diffusion controlled limits and drug/CD complexes are continuously being formed and broken apart [58]. The value of $K_{1:1}$ is most often between 50 and 2000 M^{-1} with a mean value of 130, 490 and 350 M^{-1} for αCD, βCD and γCD, respectively [24,59–61]. Non-cyclic oligosaccharides and polysaccharides are also known to form water-soluble complexes with lipophilic water-insoluble compounds [62–66]. Like non-cyclic oligosaccharides, it is also possible that CDs form non-inclusion complexes where, for example, the hydroxyl groups on the outer surface of the CD molecule form hydrogen bonds with the drug of interest. It has been shown that αCD forms both inclusion and non-inclusion complexes with dicarboxylic acids and that the two types of complexes coexist in aqueous solutions [67]. Likewise the acridine/dimethyl-βCD 2:1 complex is formed when a 1:1 acridine/dimethyl-βCD inclusion complex forms a non-inclusion complex with a second acridine molecule [68] and some 1:2 and 2:2 drug/CD complexes have also been shown to consist of a mixture of inclusion and non-inclusion complexes [69–71]. This could explain why the value of the equilibrium constant for complex formation is sometimes concentration dependent and why their numerical value is frequently dependant on the method applied [70,71]. However, the inclusion-type of guest/host CD complexes are probably much more common than the non-inclusion CD complexes.

2.1.1. Theoretical considerations

In all complexation processes including those associated with CDs, the measurement and knowledge of the stability or equilibrium constant (K_c) or its inverse, the dissociation constant (K_d) are crucial since these values provide an index of change of physicochemical properties that result upon host-guest binding including, for the discussion at hand, solubility. For complexation, the equilibrium constant ($K_{n:m}$) can be written:

$$m\text{CD} + nD \overset{K_{m:n}}{\rightleftharpoons} \text{CD}_m \bullet D_n \tag{1}$$

$$[a - mx][b - nx] \quad [x]$$

and

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

In addition, a dissociation constant can be defined as:

$$K_d = \frac{[a - mx]^m [b - nx]^n}{[x]} = \frac{1}{Km : n} \tag{3}$$

Most methods for determining the K values for drug–CD interactions are based on titrating a certain chemical or physical property of the guest molecule with the CD and then analyzing the concentration dependencies. Additive properties of the drug or guest molecule that can be addressed in this way include aqueous solubility (phase–solubility relationships), chemical reactivity, molar absorptivity and other spectrophotometric properties, NMR chemical shifts and other spectroscopic properties, pKa values and HPLC retention times among others [72].

2.1.1.1. Phase–solubility analysis.

Phase–solubility analysis of the effect of complexing agents on the compound being solubilized is a traditional approach to determine not only the value of the stability constant but also to give insight into the stoichiometry of the equilibrium. Experimentally, an excess of a poorly water-soluble drug (i.e. a substrate or drug, D) is introduced into several vials to which a constant volume of an aqueous vehicle containing successively larger concentrations of the CD are added. The need for excess drug is based on the desired to maintain as high a thermodynamic activity of the drug as possible. The vials are shaken or otherwise agitated at constant temperature until equilibrium is established. The suspensions are then filtered and the total concentration of the drug (D_t) determined based on appropriate analytical techniques (UV spectrophotometry, HPLC, etc). The phase–solubility profile is then constructed by assessing the effect of the CD on the apparent solubility of the drug (D). The practical and phenomenological implications of phase–solubility analysis were developed by Higuchi and Connons in their pioneering work published in 1964 [73] and as later reviewed by Connons [74]. Based on the shape of the generated phase–solubility relationships, several types of behaviors can be identified [75]. Phase–solubility diagrams fall into two major types: A and B (Fig. 4).

2.1.1.1.1. A-type profiles.

In A systems, the apparent solubility of the substrate increase as a function of CD concentration. Three subtypes have been defined: A_L profiles indicate a linear increase in solubility as a function of solubilizer concentration, A_P systems indicate an isotherm wherein the curve deviates in a positive direction from linearity (i.e. the solubilizer is proportionally more effective at higher concentrations) and

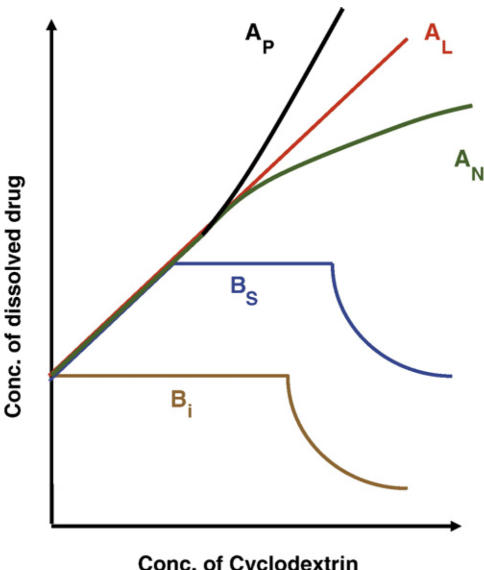


Fig. 4. Graphical representations of A and B-type phase–solubility profiles with applicable subtypes (A_P, A_L, A_N and B_S, B_I).

A_N relationships indicate a negative deviation from linearity (i.e. the CD is proportionally less effective at higher concentrations). Taken as a whole, these isotherms indicate that water-soluble complexes are being formed with solubilities higher than that of the uncomplexed substrate. A_L-type relationships are first order with respect to the CD and may be first of higher order with respect to the drug (i.e. $D\bullet CD$, $D_2\bullet CD$, $D_3\bullet CD$, etc). If the slope of the A_L isotherm is greater than unity, higher order complexes are assumed to be involved in the solubilization. Although a slope of less than one does not exclude the occurrence of higher order complexes, a one-to-one complex is often assumed in the absence of other information. A_P systems suggest the formation of higher order complexes with respect to the CD at higher CD concentrations (i.e. $D\bullet CD$, $D\bullet CD_2$, $D\bullet CD_3$, etc). The stoichiometry of the formed complexes has historically been implied by the extent of curvature of the phase–solubility profile. Thus, an isotherm best fit to a quadratic function suggest the formation of a one-to-two ($D\bullet CD_2$) complex, one best fit to a cubic function suggests a one-to-three complex ($D\bullet CD_3$), and so forth. A_N profiles have several explanations including bulk changes imparted to the solvent by the solubilizer at various concentrations (i.e. the solubilizer is acting as a chaotrope or kosmotrope or is altering the bulk properties of the media by changing its viscosity, surface tension or conductivity) and/or self-association of the solubilizer at high concentrations.

Equilibrium constants can be derived from A-type phase–solubility profiles in a number of ways. As discussed previously, the equilibrium constant for a complexation of interest is given by Eq. (2). In the original treatment of complexation, Higuchi and Connons [73] used L to denote the ligand in the complexation and S to represent the substrate. In the current review, the same formalism is applied with the exception that the substrate is termed, D (drug), and the ligand, CD (cyclodextrin). To this point, intrinsic drug solubility is given as D_o and a formed complex is represented by $D\bullet CD$.

Since

$$[D] = D_o \quad (4)$$

$$D_t = D_o + m[D_m \bullet CD_n] \quad (5)$$

$$CD_t = CD + n[D_m \bullet CD_n] \quad (6)$$

then the values for $[D_m\bullet CD_n]$, $[D]$ and $[CD]$ can be derived as:

$$[D_m \bullet CD_n] = \frac{D_t - D_o}{m} \quad (7)$$

$$[CD] = CD_t - n[D_m \bullet CD_n] \quad (8)$$

where D_o is the equilibrium solubility of the drug in the absence of the CD, D_t is the total concentration of the drug (i.e. the sum of the complexed and uncomplexed forms) and CD_t is the total concentration of the solubilizer. For equilibria that are first order with respect to the solubilizer ($n=1$), the following equation can be obtained:

$$D_t = \frac{mKD_o^m CD_t}{1 + KD_o^m} + D_o \quad (9)$$

A plot of D_t versus CD_t for the formation of $D_m\bullet CD$ should, therefore, give a straight line with the y-intercept representing D_o and the slope defined as:

$$\text{slope} = \frac{mKD_o^m}{1 + KD_o^m} \quad (10)$$

Therefore, if m is known, the K can be calculated meaning that for one-to-one complexation, i.e. $m=1$, the following graphical approach can be applied:

$$K_{1:1} = \frac{\text{slope}}{D_o(1 - \text{slope})} \quad (11)$$

It should be noted that in the circumstance where a series of complexes of the form $D\bullet CD+D_2\bullet CD+\ldots+D_m\bullet CD$ are present, an A_L-type profile would still be observed and simple phase–solubility analysis would not be able to distinguish between the simple (one-to-one) and higher order complexes. If the slope of the isotherm is greater than unity, higher order complexes are indicated and Eq. (9) can be used with substitution of various trial values of m to suggest the stoichiometry and magnitude for the equilibrium constant.

For 1:1 drug/CD complexes the complexation efficiency (CE) can be calculated from the slope of the phase–solubility diagram [76]:

$$CE = \frac{[D \bullet CD]}{[CD]} = D_0 \cdot K_{1:1} = \frac{\text{slope}}{(1 - \text{slope})} \quad (12)$$

When selecting CD or complexation conditions during formulation work it can frequently be more convenient to compare the CE than $K_{1:1}$ values since CE is less sensitive to errors related to estimation of intrinsic drug solubility.

For A_P-defined profiles, the equilibrium constants can also be calculated. For a system in which a drug interacts with two CD species:

$$K_{1:1} = \frac{[D \bullet CD]}{[D][CD]} \quad (13)$$

$$K_{1:2} = \frac{[D \bullet CD_2]}{[D \bullet CD][CD]} \text{ or } \frac{[D \bullet CD_2]}{[D][CD]^2} \quad (14)$$

where the mass balance equations are given by:

$$D_t = [D] + [D \bullet CD] + [D \bullet CD_2] \quad (15)$$

$$[D] = D_o \quad (16)$$

$$CD_t = [CD] + [D \bullet CD] + 2[D \bullet CD_2] \quad (17)$$

which when combined gives:

$$D_t = \frac{CD_t[K_{1:1}D_o + K_{1:1}K_{1:2}D_o[CD]]}{1 + K_{1:1}D_o + 2K_{1:1}K_{1:2}D_o[CD]} + D_o \quad (18)$$

This equation indicates that a plot of D_t versus CD_t will give a graph with an y-intercept of D_o and a slope which is increasing as function of [CD] yielding the observed curvilinear

representation. The above Eq. (18) can be converted into the following quadratic relationship:

$$D_t = D_o + K_{1:1}D_o[\mathrm{CD}] + K_{1:1}K_{1:2}D_o[\mathrm{CD}]^2 \tag{19}$$

indicating that a plot of $[D_t]$ versus $[\mathrm{CD}_t]$ fitted to the quadratic relationship will allow for the calculation of $K_{1:1}$ and $K_{1:2}$. Note that at low CD concentrations, $[\mathrm{CD}_t]$ is sometimes used as an estimate for [CD] meaning that a plot of the CD concentration versus drug solubilized can be used to estimate the K values. A linear form of this equation can also be derived:

$$\frac{D_t - D_o}{\mathrm{CD}_t} = K_{1:1}D_o + K_{1:1}K_{1:2}D_o\mathrm{CD}_t \tag{20}$$

Theoretically, higher order complexation can be further examined with higher order curve fitting meaning that the same formalism can be used when $K_{1:3}$, $K_{1:4}$,…, $K_{1:m}$ values are present (the cubic equation is given as an example):

$$D_t = D_o + K_{1:1}D_o[\mathrm{CD}] + K_{1:1}K_{1:2}D_o[\mathrm{CD}]^2 + K_{1:1}K_{1:2}K_{1:3}D_o[\mathrm{CD}]^3 \tag{21}$$

As suggested above, total CD concentration can be used to estimated CD_t however there are limitations to this approach. Specifically, the use of the simple polynomial to estimate the stability constants is not possible when a significant part of the total CD concentration $[\mathrm{CD}]_t$ is present as bound CD as this leads to systematic errors in the calculated stability constants. One method which avoids these errors was provided by Higuchi and Kristiansen in which they calculated the free CD concentration [77]. The first step in this process is to assume that all CD in present in the complexed form:

$$[\mathrm{CD}] = [\mathrm{CD}_t] - (D_t - D_o) \tag{22}$$

meaning (from Eq. (20)):

$$\frac{D_t - D_t}{\mathrm{CD}_t - (D_t - D_o)} = K_{1:1}D_o + K_{1:1}K_{1:2}D_o[\mathrm{CD}_t - (D_t - D_o)] \tag{23}$$

Estimates for K values are then generated and values of the [CD] determined using the equation and exact values for $[D_o]$ and $[\mathrm{CD}_t]$:

$$[\mathrm{CD}] = \frac{-(K_{1:1}D_o + 1) + \left((K_{1:1}D_o + 1)^2 + 8K_{1:1}K_{1:2}D_o[\mathrm{CD}_t]\right)^{\frac{1}{2}}}{4K_{1:1}K_{1:2}D_o} \tag{24}$$

Values for [CD] are then applied to Eq. (20) to generated improved estimates of the equilibrium constants and this iteration continues until constant values for K are obtained. This approach is easily incorporated into a least-square regression-based computer program.

While these paradigms allowed for the calculation of $K_{1:1}$ and $K_{1:2}$ values, the determination of higher order complexes is not possible with this formalism. To overcome these limitations, Peeters et al. suggested a non-linear optimization approach using a similar strategy as that of Kristiansen and Higuchi wherein trial values of the stability constants (K's) are first obtained by numerically fitting a appropriate polynomial using the total CD concentration as the independent variable [78]. The rough estimates are then used to calculate the solubility of the drug of interest as a function of the both the bound and free CD concentration at each solubility point using an exact solution to the equation. The differences between the calculated solubility and the experimentally derived data are then minimized by varying the values of the stability constants using the iterative approach of Nelder–Mead based on least-square regression. Analysis of the residuals between calculated and experimental solubility data are then used to assess goodness of model fit.

In many of these approaches, intrinsic drug solubility D_o is derived from the y-intercept of the phase–solubility relationship. Loftsson et al. pointed out that generally the y-intercept values derived from phase–solubility analysis (or $[D]_{int}$) are good estimates for D_o but only in cases where the solubility of the drug is ≥ 1 mM [76]. When the drug is less soluble than 0.1 mM significant deviation is observed and in most cases the $[D]_{int}$ value underestimated the observed intrinsic solubility. The exact cause for this deviation is not known but non-ideal behavior of water has been suggested as one possibility. Other contributing factors may be self-association of drug molecules or interaction of the drug with an excipient since these equilibria will reduce the availability of the drug for complex formation. In any case, these findings suggest that the use of $[D]_{int}$ for D_o in phase–solubility analysis will result in an overestimation of the K_c or $K_{1:1}$ values. In extreme cases, $[D]_{int}$ can be negative giving rise to a negative K value which is not possible. This situation has been observed for highly lipophilic compounds and an explanation for the negative solubility value is usually related to drug self-association as in the case of cinnarizine. Loftsson et al. has used the concept of A_L^- phase–solubility analysis (a negative deviation from linearity at low CD concentration) to define these occurrences [76].

2.1.1.1.2. B-type profiles. Type B phase–solubility profiles are indicative of the formation of complexes with limited water solubility and are traditionally observed with naturally occurring CDs, especially βCD. Two subclasses have been described including B_S and B_I systems. B_S-type isotherms have been interpreted in the following manner [73,74]: as the CD concentration increases, a soluble complex forms which increase the total solubility of the substrate. At a particular point in this solubilization process, the maximum solubility of the drug is achieved which is the sum of D_o plus any drug solubilized in the form of the CD complex. Additional CD generates additional complex which precipitates but so long as solid drug remains, dissolution and complexation can occur to maintain the value of D_t. During this plateau phase, the following equilibrium is assumed to occur:

$$\mathrm{Drug}_{Solid} \overset{D_o}{\leftrightarrow} \mathrm{Drug}_{Sol} + \mathrm{CD} \overset{K}{\leftrightarrow} \mathrm{Drug} \bullet \mathrm{CD}_{Sol} \overset{D_c}{\leftrightarrow} \mathrm{Drug} \bullet \mathrm{CD}_{Solid} \tag{25}$$

At some point, all of the solid drug will have been consumed in the above described process and further addition of the CD results

in the formation of additional insoluble inclusion complex which precipitates and further depletes the total drug concentration, $[D]_t$. Finally, the solubility observed in the systems is associated with the solubility of the precipitated complex (D_c). If the same complex which forms in the ascending portion the phase–solubility profile precipitates in the plateau phase, the increase in the drug concentration from D_o to the plateau should equal D_c. Note that this may not always be the case as multiple complexes may form in B-type systems. Importantly, the Gibbs phase rule indicates that only two phases can exist in the plateau segment of the diagram meaning that only one discrete complex may precipitate at any given point of the phase–solubility profile [73]. The stoichiometry of complexes formed from B_S-type solubility isotherms can be determined in several ways. The precipitated complex can be chemically analyzed to give information on the molar relationships between the drug and CD (i.e. the D and CD components, respectively). In addition, the length of the plateau region may be used to infer the complex order. This is possible since the amount of CD represented by the plateau is equal to that associated with the complex and the amount of drug associated with the complex is equal to the undissolved drug at the isotherm inflection (from the initial to the plateau segments). The drug content of the complex is therefore the initial amount of drug present minus the amount of drug solubilized at the isotherm infection point. The ratio of $[D]$/[CD] gives the corresponding complex stoichiometry.

The B_I systems are similar in form to the B_S profiles except that the complexes being formed are so insoluble that they do not give rise to the initial ascending component of the isotherm. Estimation of stability constants based on B-type phase–solubility relationships is also possible here. The initial ascending portion of a B_S-type isotherm can be analyzed with the same techniques used to assess A_L-type systems through the use of Eq. (11). It is also possible to calculate a K value from the descending portion of B_S profiles assuming the stoichiometry of the system is known. At some intermediate point in the descending concentration curve:

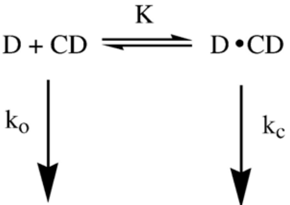
$$D_x = [D] + m[D_m \bullet \mathrm{CD}_n] \quad (26)$$
$$\mathrm{CD}_x = [\mathrm{CD}] + n[D_m \bullet \mathrm{CD}_n] \quad (27)$$

Since the aqueous solubility of the complex can be defined and since the concentration of the complex in the descending portion of the curve is constant, the following equation can be derived:

$$K_{m:n} = \frac{D_c}{[D_x - mD_c]^m[\mathrm{CD}_x - nD_c]^n} \quad (28)$$

Values for D_c can be estimated graphically, or for one-to-one complexes, as the difference between D_o and the isotherm inflection point between the ascending and plateau components.

2.1.1.2. Equilibrium constant determination based on chemical reactivity. Methods based on chemical kinetics for determining equilibrium constants have been derived since CDs can enhance or retard the rate of various chemical processes [20,60,79–82]. Due to saturation kinetics, the observed first-order rate constants for a reaction (k_{obs}) asymptotically approaches a maximum (catalysis) or minimum (inhibition) value with increasing CD concentration. The concentration dependence of the k_{obs} can be used to derive both the value of the stability constant, K_c as well as that of the first-order rate constant for the reaction of the included compound (k_c), by methods analogous to Michaelis–Menton analysis. For the formation of a one-to-one complex, the following scheme may be applied:



where k_o represent the first-order rate constant in the absence of CD. Thus, if $k_c < k_o$, the larger the values for the equilibrium constant, K, the greater will be the stabilization of the drug in question. For the above equilibrium:

$$K = \frac{[D \bullet \mathrm{CD}]}{[D]([\mathrm{CD}] - [D \bullet \mathrm{CD}])} \text{ or } \frac{[D \bullet \mathrm{CD}]}{[D][\mathrm{CD}]} \text{ for} [\mathrm{CD}] >> [D] \quad (29)$$

meaning that for the simplifying assumption to apply, the CD concentration should be at least ten-fold higher than the drug concentration. The disappearance in drug concentration is then given by:

$$-\frac{d[D_t]}{dt} = \left[k_o \frac{1}{1 + k[\mathrm{CD}]} + k_c \frac{K[\mathrm{CD}]}{1 + K[\mathrm{CD}]}\right][D_t] \quad (30)$$
$$-\frac{d[D_t]}{dt} = k_{obs}[D_t] \quad (31)$$
$$k_{obs} = \frac{k_o + k_c K[\mathrm{CD}]}{1 + K[\mathrm{CD}]} \quad (32)$$

Meaning that the first-order rate constant for drug degradation is a weighted average of k_o and k_c. The above equation can then be rearranged into several formats including those of Lineweaver–Burk (Eq. (33)), Colter (Eq. (34)) or Eadie (Eq. (35)). These equations take the following forms:

$$\frac{1}{k_{obs} - k_o} = \frac{1}{K(k_o - k_c)} \bullet \frac{1}{[\mathrm{CD}_t]} + \frac{1}{k_c - k_o} \quad (33)$$

$$\frac{[\mathrm{CD}_t]}{k_{obs} - k_o} = \frac{1}{k_c - k_o} \bullet [\mathrm{CD}_t] + \frac{1}{K(k_c - k_o)} \quad (34)$$

$$k_{obs} - k_o = -\frac{1}{k_c} \bullet \frac{(k_{obs} - k_o)}{[\mathrm{CD}_t]} + (k_c + k_o) \quad (35)$$

Using Lineweaver–Burk analysis (Eq. (33)) as an example, a plot of $1/(k_o - k_{obs})$ versus $1/[\mathrm{CD}]_t$ will give rise to a linear relationship assuming a one-to-one complex forms with a y-intercept of $1/(k_c - k_o)$ and a slope of $1/K(k_c - k_o)$ from which values of k_c and K can be derived [82]. The kinetic approach for assessing equilibrium constants is often the only method available for compounds that are poorly stable in aqueous media.

2.1.1.3. Spectrophotometric and spectroscopic methods for determining complexation constants. Spectrophotometric, spectroscopic or fluorescence methods are useful to determine the value of K if the complexation event induce changes in the compound spectra as a function of the guest-host interaction [83–85]. These changes generally reflect an alteration in the microenvironment of the drug. For UV and related processes, the changes observed are similar to those associated with dissolving the drug in a solvent of decreased polarity. These observations have been interpreted to mean that a chromophore of the drug is transferred from a more polar to a less polar environment. Titration of these optical property changes can give information on the stability constant. To this point, the equation of Benesi–Hildebrand (Eq. (36)) or Scott (Eq. (37)) can be of use:

$$\frac{L}{\Delta A}=\frac{1}{K[D_t]\Delta\varepsilon}\bullet\frac{1}{[\mathrm{CD_t}]}+\frac{1}{[D_t]\varepsilon} \quad (36)$$

$$\frac{[D_t][\mathrm{CD_t}]L}{\Delta A}=\frac{1}{\Delta\varepsilon}\bullet[\mathrm{CD_t}]+\frac{1}{K\Delta\varepsilon} \quad (37)$$

where ΔA is the difference in absorbance between the drug in the absence and presence of the CD at a particular wavelength, $\Delta\varepsilon$ is the difference in the molar absorptivities between the free and included drug and L is the path length. Thus (for the Benesi–Hildebrand Eq. (36)) a plot of $1/\Delta A$ versus $1/[\mathrm{CD}]_t$ should give a straight line (for a one-to-one complex) with the ratio of the intercept to slope generating the K value [86,87]. In addition to changes in absorbance, changes in molar ellipticity (i.e. associated with changes in the circular dichroism spectrum) may also be used as an additive properties to estimate the equilibrium constant. Circular dichroism and related technologies are also useful in detecting complex formation since inclusion of achiral drugs into chiral CDs result in the an induced CD spectrum (i.e. manifestation of extrinsic Cotton effects) [22,23].

NMR spectroscopy has also been extensively applied to the calculation of stability constants [88–90]. In addition to quantitative and qualitative information on complex formation, NMR can be used to probe the solution geometry of CD-based complexes as well as give kinetic information on their association and dissociation. Parameters that are important in this regard include chemical shifts (δ), coupling constants, nuclear Overhauser effects (NOE) and spin-spin (τ_1) and spin-lattice (τ_2) relaxation time.

Because of the molecular configuration of the CD torus, hydrogens attached to carbon 3 and 5 of the component glucose residues (i.e. H3 and H5) are situated in the cavity interior and are sensitive to compression shifts associated with drug interaction. These interactions, especially those related to aromatic ring complexation, leads to anisotropic shielding of the CD signals with a resulting upfield displacement of the resonances. Protons present in the exterior of the torus (i.e. H1, H2 and H4) are generally unaffected by complexation. That means that titration of the upfield shifts of the H3 and/or H5 signals can be used as an additive property to determine the complexation equilibrium constants. Since the H5 protons are deeper in the cavity than the H3 proton, complexes in which significant shifts are observed for both the H3 and H5 are interpreted to identify the formation of a “deep” inclusion complex while significant shifting of only the H3 signal may suggest the formation of a “shallow” complex. Since complexation will affect both the protons of the CD cavity as well as the complementary protons of the included drug, changes in chemical shifts of both the CD and drug may be used to determine the binding constants. The Benesi–Hildebrand Eq. (36) can be modified and adapted to use NMR shift data as indicated below:

$$\frac{1}{\Delta\delta}=\frac{1}{K\Delta\delta_{\max}}\bullet\frac{1}{[\mathrm{CD_t}]}+\frac{1}{\Delta\delta_{\max}} \quad (38)$$

where $\Delta\delta$ is the change in chemical shift for a CD (or drug) proton upon complexation and $\Delta\delta_{\max}$ is the limiting change in chemical shift at infinite CD concentration as defined below [91,92]:

$$\Delta\delta=\frac{[D\bullet\mathrm{CD}]}{[D_t]}\Delta\delta_{\max} \quad (39)$$

2.1.1.4. The use of other additive properties to assess stability constants. Other properties to assess complexation include the use of calorimetry [93,94], again using the formalism of Benesi–Hildebrand model as indicated below:

$$\frac{1}{\mu V}=\frac{1}{\mu V_{\max}K[\mathrm{CD}]}\bullet\frac{1}{[D]}+\frac{1}{\mu V_{\max}[\mathrm{CD}]} \quad (40)$$

where μV is microvolt and $\mu V_{\max}$ is the limiting microvolt change at infinite CD concentration. In this case, a plot of $1/\mu V$ versus $1/[D]$ at constant CD concentration will generate the K value, again, by dividing the intercept by the slope.

Potentiometric methods assess the effect of complexation on change of acidity or basicity of the complexed material [95,96]. This method is based on the finding that CDs tend to bind free acids and bases more strongly than the respective ionized species. As a consequence, CDs suppress ionization and increase the pKa for acids while decreasing the pKa for bases. For a one-to-one complex:

Drug-H + CD $\underset{}{\overset{K_d}{\rightleftharpoons}}$ [Drug-H • CD]

K_a (Drug-H + CD ⇌ Drug⁻ H⁺ + CD); K_a' ([Drug-H • CD] ⇌ [Drug⁻ • CD] + H⁺)

Drug⁻ H⁺ + CD $\underset{K_{ion}}{\rightleftharpoons}$ [Drug⁻ • CD] + H⁺

where K_a and K_a' are the dissociation constants for the free acid and the included acid, respectively an K_d and K_{ion} are the dissociation constants (i.e. $1/K_c$) for the non-ionized and ionized species, respectively. Based on this equilibrium, the following equation can be derived:

$$\frac{[\mathrm{CD_t}]}{[\mathrm{H^+}]_{hc}-[\mathrm{H^+}]_{ho}}=\frac{K_d}{K_a'(K_d-K_{ion})}[\mathrm{CD_t}]+\frac{K_d^2}{K_a'(K_d-K_{ion})} \quad (41)$$

where $[H^+]_{hc}$ and $[H^+]_{ho}$ are the hydrogen ion concentrations at the half neutralization point in the presence and absence of the CD, respectively. A plot of $[CD_t]/([H^+]_{hc}-[H^+]_{ho})$ versus $[CD_t]$ should give a straight line with the ratio of the intercept to slope giving the K_d. Further, the K_a' and the K_{ion} can be determined using the following equations:

$$K_a{}' = \frac{K_d}{\text{intercept}} + K_a \tag{42}$$

$$K_{ion} = \frac{K_a K_d}{K_a{}'} \tag{43}$$

The differential ability of drugs and drug–CD complexes to partition between aqueous and organic phases also provides an interesting approach to estimating stability constants [97]. An information rich value often used in the pharmaceutical sciences is the log of the octanol-water partition coefficient (log P). In addition to providing data on biophase distribution, the partitioning of drugs and their CD complexes has been used to calculate the binding constants, K. Having said that, this approach is complicated by the formation of complexes between octanol and the CD species and the fact that increasing saturation of CD binding sites with increasing drug concentrations must be accounted for in any exact treatment of phase distribution. Masson et al. derived an effective approach for using octanol-water partitioning approaches to determine stability constants [97]. Based on Eq. (11), the slope of the phase–solubility isotherm for the interaction of a drug and CD in a medium saturated with respect to the drug can be assessed. If two components (i.e. a drug and octanol) are present and if a one-to-one complex forms for each of the two compounds, the following equation can be obtained:

$$[D_A] = [D_{o(A)}] + \frac{K_{1:1(A)} D_{o(A)} [CD_t]}{1 + K_{1:1(B)} D_{o(B)} + K_{1:1(A)} D_{o(A)}} \tag{44}$$

where $K_{1:1(A)}$ and $K_{1:1(B)}$ are the equilibrium constants for the interaction of compound A and B with the CD, $D_{o(A)}$ and $D_{o(B)}$ is the intrinsic solubility for compounds A and B. The slope of the resulting phase–solubility profile for compound A is then given by:

$$\text{Slope}_A = \frac{K_{1:1(A)} D_{o(A)}}{1 + K_{1:1(B)} D_{o(B)} + K_{1:1(A)} D_{o(A)}} \tag{45}$$

meaning that the equilibrium constant can be solved for by:

$$K_{1:1(A)} = \frac{1}{D_{o(A)}} \bullet \frac{(1 + K_{1:1(B)} D_{o(B)})\text{Slope}_A}{1 - \text{Slope}_A} \tag{46}$$

The partition coefficient, P, is given by

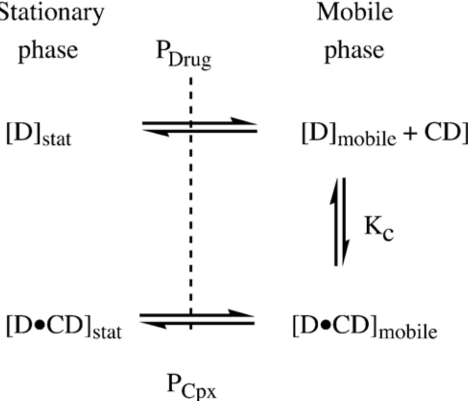
$$P = \frac{[D]_{oct}}{[D]_{aq}} \tag{47}$$

where the $[D]_{oct}$ and $[D]_{aq}$ refer to the concentration of the drug in the octanol and aqueous phase, respectively. The apparent partition coefficient, P_{app}, is then provided by the following equation:

$$P_{app} = \frac{[D]_{oct}}{[D_t]_{aq}} = \frac{[D]_{oct}}{[D]_{aq} + [D \bullet CD]_{aq}} \tag{48}$$

where $[D \bullet CD]_{aq}$ is the concentration of the formed one-to-one complex in the aqueous phase.

Stability constants can also be determined using HPLC-based methodologies [98–100]. Addition of CD to the mobile phase decreases the retention time of a guest drug depending on the magnitude of the equilibrium constant. In this process, the following equilibrium is representative for a one-to-one complex:



In this representation, P_{Drug} and P_{Cpx} are the distribution ratios for the free drug and the complex, respectively, as described below:

$$P_{Drug} = \frac{[D]_{stat}}{[D]_{mobile}} \tag{49}$$

$$P_{Cpx} = \frac{[D \bullet CD]_{stat}}{[D \bullet CD]_{mobile}} \tag{50}$$

where the subscripts stat and mobile represent the concentration of the specified material in the stationary or mobile phases. The apparent distribution ratio (P_{obs}) for the drug in the presence of a particular concentration of CD is given as:

$$P_{obs} = \frac{[D]_{stat} + [D \bullet CD]_{stat}}{[D]_{mobile} + [D \bullet CD]_{mobile}} \tag{51}$$

Combining these relationships and applying chromatographic retention times, the following relationship can be obtained:

$$\frac{[CD]_{mobile}}{t_o{}' - t_{obs}} = \frac{1}{t_o{}' - t_c}[CD]_{mobile} + \frac{1}{K_c(t_o{}' - t_c)} \tag{52}$$

where t_o', t_c and t_{obs} are the retention times for the drug, the drug•CD complex (determined as it limiting value) and the retention time for the drug at a specific CD concentration, respectively. A plot of $[CD]_{mobile}/(t_o' - t_{obs})$ versus $[CD]_{mobile}$ will give a linear relationship for a one-to-one complex from which both the K_c and the t_c can be derived. The HPLC approach has the advantage of requiring small sample sizes and the fact that complexation can be detected even if there is no change in optical properties upon complexation.

A variety of other methods are also available including stop-flow spectrophotometry and ultrasonic relaxation [101] which

can give the kinetic k_{on} and k_{off} rates. The ratio of the two rate constants gives the thermodynamic K_c value:

$$K_c = \frac{k_{on}}{k_{off}} \quad (53)$$

Equilibrium constants determined kinetically were found to be in good experimental agreement with those determined under equilibrium conditions. Other approaches which have been applied include conductmetric, polarimetric and positron annihilation methods. In recent years, the use of membrane-based equilibrium techniques have been applied as have affinity capillary electrophoresis [102].

In addition to methods for determining the complexation constants, monitoring of additive properties can also give insight into the stoichiometry of the complex. This is often preformed using the method of continuous variation or the construction of Job plots in which the change in the additive properties (e.g. absorbance, ellipticity, chemical shift, etc) is assessed as a function of complexation [103,104]. The extent of change of the additive property in the system will be a weighted average of the various species present in solution including the drug and the drug•CD complex or complexes. Varying the drug concentration in a solution where the sum of the drug and CD concentration is constant will cause the additive property observed to pass though a maximum or minimum. This maximum or minimum corresponds to the highest concentration of the complex and therefore the mole fraction at which this occurs is indicative of the stoichiometry of the complex in solution. Plots of this type can also be used to assess stability constants via simplex optimization approaches.

The application of these multifarious techniques to characterize solubilization processes involving CDs are found throughout the literature. As mentioned in Table 1, they have contributed to the market introduction of pharmaceutical products. Numerous monographs and reviews are available on the assessment and calculation of stability constants ([20–23,25,34] and a dedicated issues of this journal: Adv. Drug Deliv. Rev. Vol. 36, Issue 1). Table 5 gives a summary of recent publications including which methods were applied to determine the K_c as well as the complex stoichiometry.

2.1.2. Thermodynamics and driving forces for complexation

The thermodynamic parameters for CD complexation, i.e. the standard free energy change (ΔG), the standard enthalpy change (ΔH) and the standard entropy change(ΔS), can be obtained from the temperature dependence of the stability constant of the CD complex [105]. The thermodynamic parameters for several series of drugs and other compounds have been determined and analyzed [20,106–111]. A number of forces have been examine for their role in driving complex formation including electrostatic interactions, van der Waals contributions, hydrogen bonding, release of conformational strain, exclusion of high energy water bound in the CD cavity and charge–transfer interactions [112–115]. The complex formation is almost always associated with a relatively large negative ΔH while the ΔS can be either positive or negative. Also, the complex formation is largely independent of the chemical properties of the guest (i.e. drug) molecules. The association of binding constants with substrate polarizability suggest that van der Waal's forces are important in the complex formation [116]. Classically, hydrophobic interactions are associated with a slightly positive ΔH and large positive ΔS indicating that they are entropy-driven in contrast to CD complexation, which is enthalpically driven. Based on the relatively apolar environment of the CD cavity, it may be expected that the water molecules situated therein do not have a full complement of hydrogen bonds and are at higher energy than those in the bulk media. Liberation of these "enthalpy-rich" molecules may represent a driving force in this context. Conversely, some have argued that the while cavity-bound water may be of higher energy, it may also be more entropically flexible due to the absence of hydrogen bonding [117]. Thus, while release of cavity-bound water may be associated with a negative enthalpy, its overall free energy contribution may be small. It has been observed that for a series of guests there tends to be a relationship between enthalpy and entropy, with increasing enthalpy related with less negative entropy values [22]. This effect, termed enthalpy–entropy compensation, is often correlated with solvent restructuring as a driving force in complex formation. A theory to this point, derived by Grunwald and Steel, is consistent with solvent restructuring as the primary explanation of entropy–enthalpy compensation and holds that these effects are more important in the interaction of CDs with lipophiles than with hydrophilic compounds [117]. All in all, there appears to be multiple driving forces for complex formation which may be exerted simultaneously. While multivariate QSAR analysis can provide useful information on the driving force for a particular drug–CD interaction [118], these approaches are system-specific and not generally applicable. CD complex formation usually results from a combination of electrostatic interactions, van der Waal's forces, hydrogen bonding and charge–transfer interaction. The observed enthalpy–entropy compensation dimension suggests that expulsion of high energy water and relief of conformation strain may play a lesser role in driving complex formation [117].

2.2. Self-association of CDs and CD complexes

While CD based interactions are related to their ability to form dynamic inclusion complexes, this single interaction fails to explain all observation made when using CDs as a solubilizer especially when phase–solubility analysis is the analytical endpoint [70,71]. In these analyses, the drug is saturated in the CD solution providing for the possibility of non-ideal solution effects. When stability constants derived from phase–solubility techniques are compared to those derived using other approaches (i.e. NMR, spectrophotometry or potentiometric titrations), significant differences have been observed. Case examples include carbamazepine and phenylbarbituric acid. These compounds may interact with the CD exterior to from non-inclusion complex analogous to the interaction of drugs with non-cyclic oligosaccharides (see Section 2.1). Finally, current models of CD complexation assume that each drug–CD entity interacts independently of all other such complexes. This

Table 5
Recent publication involving stability constant (K_c) calculations for cyclodextrins

Drug/compound	Cyclodextrin *	K_c/complex determined using **	Solubility enhancement	Reference
Meloxicam	αCD, βCD, γCD, HPβCD	PS, pKa	1.2 to 3-fold	Abdoh et al. 2007 [173]
Imatinib	βCD, RMβCD	PS, NMR, pKa, ESI-MS	10-fold	Beni et al. 2007 [174]
Bupivacaine	HPβCD	PS, HPLC	1.5 to 4.5-fold	Moraes et al. 2007 [175]
Omeprazole	βCD, MβCD	PS, NMR, MM	1.7 to 3.4-fold	Figueiras et al. 2007 [176]
Sidenafil	βCD, HPβCD, γCD, αCD	PS, NMR, MM	1.6 to 3.2-fold	Al Omari et al. 2006 [177]
Progesterone	HPβCD, HPγCD, PMβCD, SBEβCD	PS	4-3600-fold	Lahiani-Skiba et al. 2006 [178]
Ginseng saponin	βCD, HPβCD	PS	40 to 80-fold	Lee et al. 2006 [179]
Naftifine	αCD, βCD, γCD, MβCD, HPβCD	PS	0 to 21-fold	Uzqueda et al. 2006 [180]
Disoxaril	DMβCD	PS	3800-fold	Ventura et al. 2006 [181]
Ozonide anti-malarials	βCD, SBEβCD	PS, μCAL, NMR, ESI-MS, MD		Perry et al. 2006 [182]
Valsartan	HPβCD	PS, NMR	18-fold	Cappello et al. 2006 [183]
Nelfinavir	βCD	PS		Torne and Vavia 2006 [184]
Gossypol	βCD	PS, FS	2.5-fold	Shen et al. 2006 [185]
Celecoxib	αCD, βCD, γCD, HPβCD	PS	2.5 to 20-fold	Al Omari et al. 2006 [186]
Valdecoxib	βCD	PS, MM	3.5-fold	Jadhav et al. 2006 [187]
Benzocaine	βCD	PS, NMR, FS	3-fold	Pinto at al. 2005 [188]
Hesperetin	HPβCD	PS, FS	10-fold	Tommasini et al. 2005 [189]
Flurbiprofen	βCD, HEβCD, MβCD	PS, MEM, ACE, MM	8-160-fold	Cirri et al. 2005 [102]
Quercetin	βCD, HPβCD, SBEβCD	PS, CK, NMR, MM	21-55-fold	Zheng et al. 2005 [190]
Retinoic Acid	αCD, HPβCD	PS, MM	5-50+-fold	Yap et al. 2005 [191]
Valdecoxib	βCD, HPβCD, SBEβCD	PS, NMR, CD	60-250-fold	Rajendrakumar et al. 2005 [192]
Prednisolone	αCD, βCD, γCD, HPβCD, SBEβCD, G1βCD, G2βCD, SBEγCD	PS, NMR	10-90-fold	Larsen et al. 2005 [193]
Triclosan	βCD, HPβCD, RMβCD	PS	80-1500-fold	Loftsson et al. 2005 [194]
Risperidone	αCD, βCD, γCD, HPβCD	PS	0 to 70-fold	El-Barghouthi et al. 2005 [195]
Celecoxib	βCD	PS, NMR	5-fold	Sinha et al. 2005 [196]
3-Hydroxyflavones	αCD, βCD	PS, NMR, UV	6-fold	Calabro et al. 2004 [197]
Triamterene	βCD	PS, NMR	3-fold	Mukne and Nagarsenker 2004 [198]
Camptothecin	HPβCD	PS	30 to 50-fold	Sætern et al. 2004 [199]
Furnidine	βCD	PS, UV, PG	3.4-fold	Yáñez et al. 2004 [200]
Celecoxib	βCD	PS, UV	2.3-fold	Rawat and Jain 2004 [201]
Furan derivative G1	βCD	PS	3.3-fold	Castro-Hermida et al. 2004 [202]
Z–Glu–Tyr and related compounds	αCD, βCD, γCD	μCAL, NMR		Yamamura et al. 2004 [203]
Ampicillin	βCD, HPβCD	μCAL, NMR, MD		Aki et al. 2004 [204]
Rofecoxib	βCD, SBEβCD	PS, NMR, CD	2-fold	Rajendrakumar et al. 2004 [205]
Furosemide	HPβCD	PS, NMR	11-fold	Vlachou and Papaïoannou 2003 [206]
Natamycin	βCD, γCD, HPβCD	PS	16 to 152-fold	Koontz and Marcy 2003 [207]
Bisabolol	βCD	PS	1.7-fold	Waleczek et al. 2003 [208]
Acitretin	HPβCD, RMβCD	PS,MM	>1000-fold	Liu et al. 2003 [209]
Fentanyl	HPβCD, SBEβCD, G2βCD	PS	1-10-fold	Holvoet et al. 2003 [210]
Rofecoxib	βCD	PS, UV	3-fold	Rawat and Jain 2003 [211]
Artemisinin	HPβCD	PS, MM	15-fold	Illapakurthy et al. 2003 [212]
Ketoconazole	HPβCD, MβCD	PS	320 to 900-fold	Taneri et al. 2003 [213]
Carprofen	HPβCD	PS, NMR, CK	52-fold	Chen et al. 2003 [214]
Diclofenac	βCD	PS, CD	5-fold	Barbato et al. 2003 [215]

* αCD — α-cyclodextrin, βCD — β-cyclodextrin, γCD — γ-cyclodextrin, HPβCD — hydroxypropyl-β-cyclodextrin, HEβCD — hydroxyethyl-β-cyclodextrin, MβCD — methylated β-cyclodextrin, DMβCD — dimethyl-β-cyclodextrin, RMβCD — randomyl methylated β-cyclodextrin, PMβCD — permethylated β-cyclodxtein, SBEβCD — sulfobutylether-β-cyclodextrin, SBEγCD — sulfobutyl-γ-cyclodextrin, G1βCD — glucosyl-β-cyclodextrin, G2βCD — maltosyl-β-cyclodextrin.
** PS — phase–solubility, NMR — nuclear magnetic resonance spectroscopy, MM — molecular mechanics, pKa — potentiometric titration, ESI-MS — electrospray mass sepctrometry, HPLC-high performance liquid chromatrography, μCAL — microcalorimetry, MD — molecular dynamics, FS — fluorescence spectroscopy, MEM — membrane permeability, ACE — affinity capillary electrophoresis, CK — chemical kinetics, CD — circular dichroism, UV — ultraviolet spectrophotometry, PG — polarography.

is in contrast to experimental findings where evidence demonstrates that both CDs and CD complexes can aggregate [119]. In most cases, these aggregates remain small (<20 nm) and do not affect the optical properties of the solutions but in some cases they can grow such that they can be macroscopically observed. These aggregates also have been reported to be good solubilizer through either non-inclusion complex formation or via structures akin to micelles. The ability of CDs to interact in this way has been suggested for complexes of HPβCD with diflunisal and ibuprofen.

Another observation is that A_P-type profiles with CDs superficially resemble surfactant-based phase–solubility

profiles for lipophilic compounds [70,71]. This has lead to speculation that one-to-one drug–CD complexes may interact with additional drug molecules through non-inclusion mechanism to results in increased aqueous solubility. Experimental evidence for this hypothesis includes the observation that the solubility of cyclosporin A is 33% higher in HPβCD which has been pre-saturated with cholesterol than in a simple HPβCD solution [120].

2.3. Effect of additives on complexation

For a variety of reasons including isotonicity of parenteral formulations and the formulation bulk of solid dosage forms, a critical design element for the use of CD in pharmaceutical formulations is to minimize their concentrations where possible [61]. Various methods have been applied to enhance the complexation efficacy [121]. These include addition of polymers to the complexation media [122–128], drug ionization and salt formation [129,130], addition of hydroxy carboxylic acids to the complexation media [131,132], addition of volatile acids or bases to the complexation media [133], addition of organic salts [120], amino acids [134] and addition of cosolvents [135]. However, even under the best circumstances, CD complexation will result in over four-fold increase to the formulation bulk of solid dosage forms [61]. The ability of different CDs to solubilize a given drug is frequently evaluated by comparing the stability constants. For a 1:1 drug/CD complex, the stability constants ($K_{1:1}$) can be determined using Eq. (11), that assumes that the intrinsic solubility (D_0) is equal to the y-intercept of the linear phase–solubility profile. However, due to hydrophobic interactions of lipophilic water-insoluble drugs, the presence of multiple complex structures and non-ideality of saturated drug solutions, the value of D_0 is frequently much greater than that of suggested by the y-intercept [76]. As mentioned in Section 2.1.1.1, a better way to compare the solubilizing effects of CDs is to compare their complexation efficiency (CE) which is calculated from the slope of the phase–solubility diagram according to Eq. (12) [76,136]. The CE of selected drugs and CDs are listed in Table 6. The table also shows the effects of some water-soluble polymers on the CE. Cyclosporine A and tamoxifen have very low CE and, thus, only one out of every 90 to 250 CD molecules form a complex with the drug molecule (assuming 1:1 drug/CD complex formation). Consequently, large amounts of CD are needed to solubilize small amounts of the drug making CD solubilization of the drugs impractical. For a drug with molecular weight of 350 Da and CD of molecular weight of 1400 Da, a CE of 0.5 will result in a 13-fold increase of the dosage bulk of a solid dosage form, a CE of 0.1 would result in about 40-fold increase in the dosage bulk and CE of 0.01 in about 400-fold increase.

Addition of small amount of water-soluble polymer to an aqueous complexation medium frequently results in an increase in the CE and, consequently, is less demanding on the formulation bulk (Table 6). It is well-known that water-soluble polymer do form complexes with a wide variety of compounds [64–66,137] and that they stabilize micelles and other types of aggregates in aqueous solutions [138–140]. It is also known that water-soluble polymers can solubilize βCD and its complexes [141], and apparent stability constants between polymers and CDs, as well as between polymers and CD complexes, have been determined [142]. Quaternary complexes have also been described involving drug, CD, polymer and a fourth component such as tartaric acid [132] or magnesium ions [143]. However, such effects are highly dependent on the both the type of polymer, the drug and the CD applied [122]. For example, αCD forms inclusion complexes, i.e.

Table 6
The Complexation Efficiency (CE) and the drug:CD molar ratio in aqueous CD solutions saturated with the drug for some selected drugs and cyclodextrins

Drug [a]	pK_a [b]	Cyclodextrin [c]	Additive [d]	pH [e]	Solubility (mg/ml)		CE [h]	Molar ratio [i]
					S_0 [f]	$S_{10\%}$ [g]		
Acetazolamide (A)	7.2	HPαCD	–	W	0.64	–	0.015	1:70
		HPβCD	–	W	0.64	3.6	0.197	1:6
		HPβCD	HPMC	W	0.90	4.4	0.356	1:4
		HPβCD	CMC	W	0.59	3.6	0.209	1:6
		HPβCD	PVP	W	0.94	3.7	0.273	1:5
		HPβCD	–	6.4	1.4	3.2	0.128	1:9
		HPβCD	–	3.6	0.76	2.9	0.156	1:7
		RMβCD	–	W	0.64	7.1	0.566	1:3
		HPγCD	–	W	0.64	–	0.021	1:50
Carbamazepine (B)	7	HPβCD	–	W	0.26	6.5	0.548	1:3
		HPβCD	HPMC	W	0.33	8.0	0.829	1:2
		HPβCD	CMC	W	0.18	8.4	0.709	1:2
		HPβCD	PVP	W	0.28	8.5	0.701	1:2
		HPβCD	–	6.3	0.06	8.0	0.679	1:2
		RMβCD	–	W	0.26	6.2	0.479	1:3
Cyclosporine A		αCD	–	W	0.01	0.9	0.011	1:90
		HPαCD	–	W	0.01	0.2	0.004	1:250
		RMβCD	–	W	0.01	0.4	0.007	1:140
Finasteride		HPβCD	–	W	0.06	9.6	0.625	1:3
		RMβCD	–	W	0.06	12.3	0.708	1:2
		RMβCD	HPMC	W	0.06	11.6	0.789	1:2
		RMβCD	CMC	W	0.06	11.5	0.805	1:2
		RMβCD	PVP	W	0.06	11.6	0.844	1:2
		SBEβCD	–	W	0.06	7.2	0.678	1:2
Oxazepam (B)	1.7	HPβCD	–	W	0.05	2.1	0.109	1:10
		HPβCD	HPMC	W	0.27	2.1	0.076	1:14
		HPβCD	CMC	W	0.05	1.5	0.127	1:9
		HPβCD	PVP	W	0.10	1.4	0.115	1:10
		RMβCD	–	W	0.05	2.4	0.163	1:7
Propofol (A)	11	HPβCD	–	W	0.16	7.7	1.44	1:2
		RMβCD	–	W	0.16	10.0	2.205	2:3
		SBEβCD	–	W	0.16	6.2	4.105	4:5
Tamoxifen		HPβCD	–	W	0.00	0.2	0.004	1:250
Triazolam (B)	2.4	HPβCD	–	>3.5	0.03	0.5	0.017	1:60

[a] Proton donor (A); proton acceptor (B).
[b] pKa values from literature [222].
[c] 2-Hydroxypropyl-α-cyclodextrin (HPαCD); 2-hydroxypropyl-β-cyclodextrin (HPβCD); randomly methylated β-cyclodextrin (RMβCD); 2-hydroxypropyl-γ-cyclodextrin (HPγCD); α-cyclodextrin (αCD); sulfobutylether β-cyclodextrin sodium salt (SBEβCD).
[d] No additive (−), 0.10% (w/v) hydroxypropyl methylcellulose (HPMC); 0.25% (w/v) sodium carboxymethylcellulose (CMC); 0.25% (w/v) polyvinylpyrrolidone (PVP).
[e] Pure water (W) or aqueous buffer solution.
[f] Drug solubility in the complexation medium when no CD is present.
[g] Drug solubility in the complexation medium when 10% (w/v) is present.
[h] The complexation efficiency calculated according to Eq. (12).
[i] Calculated drug:CD molar ratio: molar ratio = 1:(CE + 1)/CE.

pseudo-polyrotaxanes, with polyethylene glycol (PEG) whereas βCD does not, and βCD forms inclusion complexes with polypropylene glycol whereas αCD does not [144]. Moreover, formation of polymer/CD inclusion complexes will frequently reduce the ability of the CD molecules to solubilized drugs through complexation.

The complexation efficiency has also been increased by ionization of the drug molecule through addition of volatile acid (i.e. acetic acid) or base (i.e. ammonia) to the aqueous complexation media of basic or acidic drugs, respectively [133,145]. The volatile acid or base is then removed during lyophilization or heating under vacuum resulting in formation of solid CD complexes of the unionized drug.

2.4. Solubilization effects related to supersaturation

As illustrated in this review, CD can increase the apparent solubility and dissolution rate of poorly water-soluble drug candidates improving their biopharmaceutical performance. The traditionally ascribed mechanisms of inclusion complex formation as well as non-inclusion complex formation accounts in large part for these effects. In some instance other mechanism may be involved. One example is the interaction of different physical forms of a drug or a drug formulation with a CD solution. Brewster et al. reported that solubilization of crystalline itraconazole in 20% w/v HPβCD in an acidic medium was associated with a day 1 solubility value of 35 mg% which increased slowly over time to give a day 28 value of 50 mg%. Conversely, amorphous itraconazole generated 1160 mg% at a day 1 measurement which slowly decreased to 200 mg% at day 28 [146]. One explanation for these observations is the possible effect of HPβCD as a stabilizer of the formed supersaturated system via nucleation and crystal growth inhibition. To this point, both HPβCD and SBEβCD were found to support the formation of supersaturated solutions of itraconazole based on a co-solvent method and further stabilized the formed supersaturated solutions over 2 h. These effects were significantly improved over those observed using surfactants and other excipients (Fig. 5). Along the same lines, Xiang and Anderson found that the generation of supersaturated solutions of a novel anti-cancer agent (Silatecan) was possible by converting a precursor to the lactone at an appropriate pH in a SBEβCD solution [147]. These systems were stable for up to three days after preparation and could be lyophilized and successfully reconstituted. Uekama et al. has also assessed the ability of CD to inhibit crystal growth in drug formulations. In a spray dried dispersion of nifedipine and HPβCD, crystal growth was minimized such that neither dissolution changes nor attenuation of oral bioavailability in dogs were observed in aged material compared to freshly prepared dispersions [148,149]. Finally Torres-Labandeira et al. found that supersaturated solutions of pancratistatin could be prepared in HPβCD solutions [145]. In their preparation procedure, the drug was treated with ammonium after which the aqueous ammoniac solution was removed by freeze-drying. Reconstitution of the powder allowed for solutions as high as 9 mg/mL to be prepared. While precipitation was observed in these samples, the use of polyethylene containers as well as HPγCD provided for significantly prolonged the latency period prior to precipitation. Other examples in which CDs may affect supersaturation are available in the literature [150–158].

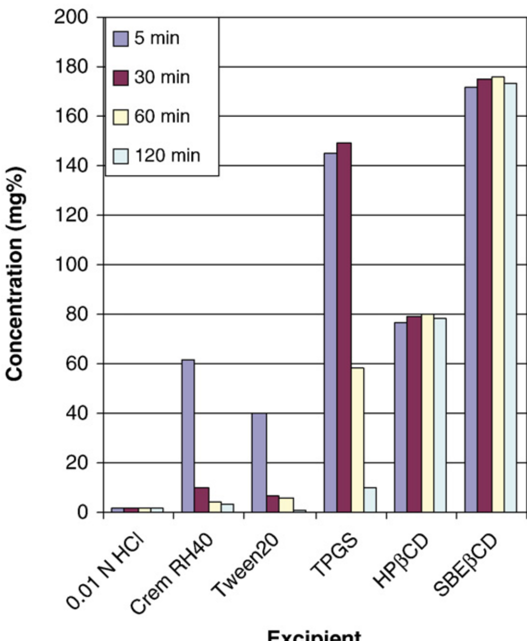


Fig. 5. Supersaturation data for itraconazole in the presence of various excipients. Equilibrium solubility at the same excipient concentration (2.5% w/v) for TPGS was 1.3 mg%, HPβCD was 3.2 mg% and SBEβCD was 19.5 mg%.

As reviewed by Macie and Grant, the actual process of nucleation is not known with any degree of certainty but a nucleus must form to serve as a center for deposition of solute from the supersaturated system and subsequent crystal growth [159]. Crystal growth is believed to take place in three steps including (1) the molecule diffuses from the bulk media to the solid crystal interface, (2) the adsorbed molecule, through a surface reaction, becomes part of the crystal lattice and the heat of crystallization released and (3), the heat of crystallization is conducted to the bulk medium.

Materials that may inhibit nucleation or crystal growth have been reported [160–163]. The mechanism of inhibition may include altering bulk properties such as surface tension or saturation solubility, changing the adsorption layer at the crystal-medium interface, selectively adsorbing to the crystal interface thereby blocking crystal growth, being adsorbed into growth layers and thereby disrupting growth layers across the surface, adsorbing into surface imperfections causing rough surfaces to become flat and/or altering the surface energy of the crystal face.

The effect of pharmaceutical polymers such as methyl cellulose (MC), hydroxypropylmethyl cellulose (HPMC) and polyvinylpyrrolidone (PVP) has traditionally been ascribed to several of the effects described above [160]. Simonelli et al. suggested that the inhibitory effect of PVP on the nucleation and growth of sulfathiazole was associated with the polymer forming a porous net around growing crystals such that crystal growth was relegated to the pores formed in the polymeric network [164]. Others have suggested that the polymers adsorb to the crystal thereby inhibiting the addition of molecular units from the bulk

solution [160]. An additional mechanism that has been brought forward is the suggestion that these polymers can solubilize the drug of interest through complexation thereby reducing the degree of supersaturation [12,165]. Raghavan et al. correlated the hydrogen bond donor and acceptor properties of hydrocortisone acetate, HMPC and PVP to explain the inhibitory effect of the polymers on drug nucleation and crystal growth [160,161]. The working theory developed included an interaction of the polymer in the unstirred water layer separating the growing crystal and the bulk media. Specifically, the polymers were postulated to hydrogen bond to growth sites on the crystal thereby blocking growth. In addition, even though these polymers may be rejected at the crystal interface, they may, for a variety of reasons, accumulate in the unstirred water layer and increase diffusional resistance. These effects were predicted to be higher for those faces of the growing crystal that have a higher hydrogen bonding potential which would result in a change in the habit of the crystals as well as their growth rates. Yokoi et al. suggested that HPMC and sugar esters (sucrose fatty acid esters) exerted differential effects on the stabilization of aqueous suspensions of cefditoren pivoxil based on the nature and extent of surface binding [166].

These suggested mechanisms for inhibition of nucleation and crystal growth may also apply to the CDs. Specifically, CD solubilize compounds by inclusion complex formation with possible contribution from non-complex based mechanisms. This increases the chemical potential of the drug in solution, increases the apparent saturation solubility and decreases the extent of supersaturation [12,158,152]. However, this effect is not often capable of accounting for the effect on the formed supersaturated solution based on the magnitude of the changes on the apparent solubility. An additional contribution may be the interaction of the CD with the growing crystal in a manner analogous to HMPC, via 1) with hydrogen bonding to sites associated with crystal growth, 2) accumulation in the unstirred water layer resulting in an increase in viscosity and hence diffusional resistance or 3) complexation of the CD with drug monomers inhibiting efficient mass transfer at the interface.

In some cases, the preparation method can promote the formation of supersaturated systems as in cases where ammonia or volatile acids are added to facilitate complexation and then remove via lyophilization [133]. Pedersen et al. suggested that supersaturation was more likely when a molecular complex was assessed as compared to physical mixtures prepared by freeze-dryng or grinding [167]. For complexes, the dissolution rate was found to be limited meaning that drug release as a function of CD concentration provides for supersaturation. The more rapid dissolution of the physical mixtures means that higher amounts of cyclodextrin are available for complexation yielding a reduction of the chemical potential (increased solutbility) of the drug in solution.

3. Conclusions

Within the pharmaceutical industry the nature of drug screening has evolved over the years such that high throughput screening techniques have become routine. These hit identification strategies put a type of evolutionary pressure on emerging drug candidates, which has led to a systematic increase in molecular weight, lipophilicity and a decrease in water solubility of lead compounds over time [168,169]. Retrospective studies in the late 1980's showed that >40% of drug failures in development could be traced to poor biopharmaceutical properties namely, poor dissolution or poor permeability [170]. Based on an analysis completed in the 2000's, this situation has improved. However poor solubility continues to impact the development of a large number of potential drug candidates [171]. These factors have had a significant impact on what is required from formulators in that the number of formulation options and by extension, excipients, has to be increased to address the larger number of challenges being presented [172]. CDs represent a true added value in this context. These starch derivatives are useful solubilizers, enabling both liquid oral and parenteral dosage forms. In addition they can increase the oral bioavailability of solids through an increase in dissolution rate secondary to increasing the apparent solubility of a compound. While inclusion complex formation is certainly the major mechanism associated with the solubilization potential of CDs, effects related to non-inclusion complexation and supersaturation may be important contributors to solubilization in certain circumstance. Many CDs including αCD, βCD, γCD, RMβCD, HPβCD and SBEβCD have become standard tools in the formulation armamentarium and are applied not only to formulation design but also to the early testing of drug candidates. Given the number of commercially available CD-containing formulations including several very recent market introductions such as Abilify ® (aripiprazole), the future for pharmaceutical application of CD as a solubilizer seems bright. The expanded use of known cyclodextrins as well the development of new derivatives continues to energize this field of study.

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