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Review

Self-assembled cyclodextrin aggregates and nanoparticles

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ABSTRACT

Cyclodextrins (CDs) are widely used as enabling pharmaceutical excipients, mainly as solubilizing complexing agents. CDs are cyclic oligosaccharides with hydrophilic outer surface and a somewhat lipophilic central cavity. In aqueous solutions CDs are able to solubilize lipophilic drugs by taking up some lipophilic moiety of the drug molecule into the central cavity, i.e. through formation of hydrophilic inclusion complexes. Recently it has been observed that that other types of CD complexes, such as non-inclusion complexes, are also participating in the CD solubilization of poorly soluble drugs. However, in aqueous solutions CDs are also able self-assemble to form nanosized aggregates that can contribute to their solubilizing properties. At low CD concentrations (at about 1%, w/v) the fraction of CD molecules forming aggregates is insignificant but the aggregation increases rapidly with increasing CD concentration. Also, formation of CD complexes can increase the tendency of CDs to form aggregates and can lead to formation of micellar-type CD aggregates capable to solubilize poorly soluble compounds that do not readily form inclusion complexes. In this article formation of CD aggregates and CD nanoparticles is reviewed with emphasis on the physicochemical properties of self-assembled CDs and CD complexes.

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1. Introduction

In its most simple form pure drug nanoparticles are being used to increase apparent aqueous solubility and oral availability of poorly soluble drugs but more sophisticated nanoparticles can be applied to target drug delivery to specific cells or tissues, or as vehicles for gene delivery, after parenteral administration (Davis and Brewster, 2004). Nanoparticulate drug delivery systems can improve drug bioavailability, reduce drug immunogenicity, modify drug metabolism, reduce drug toxicity, and increase biological half-life of drugs after systemic administration (De Jong and Borm, 2008; Sanvicens and Marco, 2008). In aqueous solutions carbohydrates and oligosaccharides self-associate to form aggregates (Patel et al., 2007). Cyclodextrins (CDs) are oligosaccharides that are used as enabling excipients in numerous marketed drug formulations. CDs are known to form nanosized aggregates in aqueous solutions and thus have the potential to develop in to sophisticated drug delivery systems. In this article the currently available literature on CD aggregates and CD nanoparticles is being reviewed with emphasis on the physicochemical properties of self-assembled CDs and CD complexes. Recently Trichard, Duchêne and Bochot reviewed dispersed systems, such as self-assembled polymeric CD nanoparticles and amphiphilic CD nanoparticles, which are not covered in this short review (Trichard et al., 2006).

Classification of particulate drug delivery systems is primarily based on their particle diameter (Fig. 1). Microparticles usually refer to particles with diameter between 0.1 and 100 μm and nanoparticles to particles with diameter between 1 and 100 nm although in drug delivery nanoparticles can have diameter up to 1000 nm (Kreuter, 1994). Particulate drug delivery systems are also classified according to their characteristics and composition. For example, liposomes, microemulsions, dendrimers, carbon nanotubes

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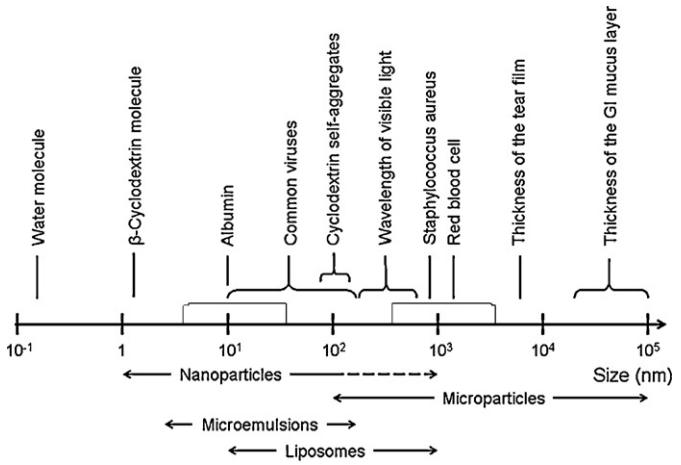


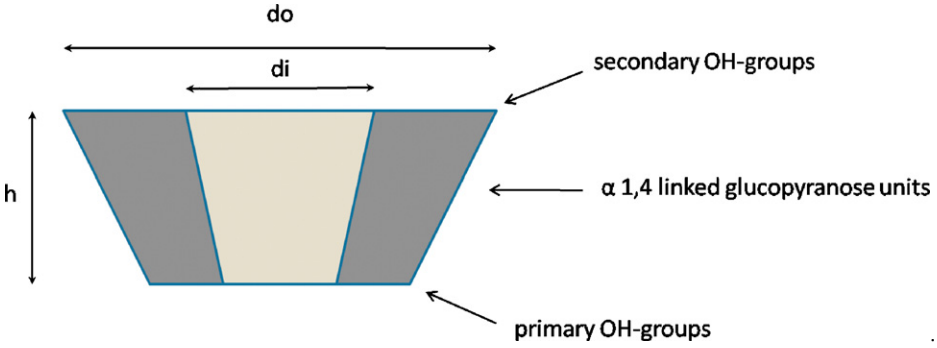
Fig. 1. The size distribution of nanoparticles, microparticles, microemulsions and liposomes in relation to various naturally occurring phenomena.

and nanoparticles are different classes or types of nanoparticulate delivery systems (Sanvicens and Marco, 2008). Liposomes are phospholipid vesicles with diameter between 50 and 100 nm with bilayer membrane structure similar to that of biological membranes while microemulsions have micellar droplets with diameter between 5 and 140 nm. Dendrimers are repeatedly branched molecules (diameter <15 nm) characterized by their central core, an internal region and numerous terminal groups. But carbon nanotubes are members of the fullerene structure family consisting of graphite sheets rolled up into cylinders with length less than 100 nm. Nanoparticles can be divided into nanospheres (e.g. drug in macromolecular matrix) and nanocapsules (e.g. macromolecular coated drug particles). The macromolecules can be natural, such as albumin, chitosan, gelatin and starch, or synthetic like polylactic acid. Still another class of particulate drug delivery systems is the self-assembled particulates that cover not only previously mentioned liposomes and microemulsions, but also non-surfactant self-assemblies like oligosaccharide nanoparticles, including CD aggregates. However, naturally occurring nanosize particles such as viruses and incidental colloidal particles are commonly excluded from the term 'nanoparticle'. Also, nanoparticle drug delivery systems should consist of at least two components, one of which is a therapeutically active ingredient (De Jong and Borm, 2008). Many nanoparticulate systems consist of particles or droplets with diameter less than 1/4 of the wavelength of the visible light (i.e. 380–750 nm) and thus dilute aqueous solutions containing such systems appear to be clear solutions to the naked eye. In contrast, microparticulate systems frequently scatter light and appear turbid. Self-assembled CD aggregates frequently have diameter of about 100 nm (Fig. 1).

2. Cyclodextrins and their chemistry

When starch is degraded by cyclomaltodextrin glucanotransferase (EC 2.4.1.19) one of several turns of the amylose helix is hydrolyzed and the ends joint together to form cyclic oligosaccharide called cyclodextrin (CD). The glucose units are linked by α-1,4-bonds and the chair formation of the glucopyranose units shapes the CD molecule into a cone with secondary hydroxyl groups extending from the wider edge and the primary groups from the narrow edge (Table 1) (Saenger et al., 1998). In aqueous solutions these hydroxyl groups form hydrogen bonds with water making CDs soluble in water. Another feature of the CD structure is the somewhat lipophilic central cavity enabling them to form guest/host type of inclusion complexes. The lipophilicity of their central cavity is comparable to an aqueous ethanolic solution (Frömming and Szejtli, 1994). Most abundant natural CDs consist of six (αCD), seven (βCD) or eight (γCD) glucopyranose units. Although the natural CDs and their complexes are hydrophilic, their aqueous solubility is rather limited, especially that of βCD. This is thought to be due to relatively strong binding of CD molecules in the crystal state (i.e. relatively high crystal lattice energy) (Loftsson and Brewster, 1996), and intramolecular hydrogen bond within the CD molecule, preventing their hydrogen bond formation with surrounding water molecules (Coleman et al., 1992). Random substitution of the hydroxyl groups, even by hydrophobic moieties

Table 1
Physicochemical properties of the natural αCD, βCD and γCD.



Property	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin
Molecular weight of anhydrous compound (dalton):	973	1135	1297
Number of glucopyranose units:	6	7	8
Number of water molecules present in the stable hydrates of the CD lattices (Sabadini et al., 2006):			
Total number:	6.4	9.6	14.2
Inside the cavity:	2	6	8.8
Approximate dimensions (nm) (Dodziuk, 2006):			
Height (H):	0.78	0.78	0.78
Inner diameter (di):	0.50	0.62	0.80
Outer diameter (do):	1.46	1.54	1.75
Solubility in water at 25 °C (mg/ml) (Sabadini et al., 2006):	129.5 ± 0.7	18.4 ± 0.2	249.2 ± 0.2
$K_{1:1}$ (population mean ± standard deviation, 25 °C) (Connors, 1995, 1997):	130 ± 8	490 ± 8	350 ± 9

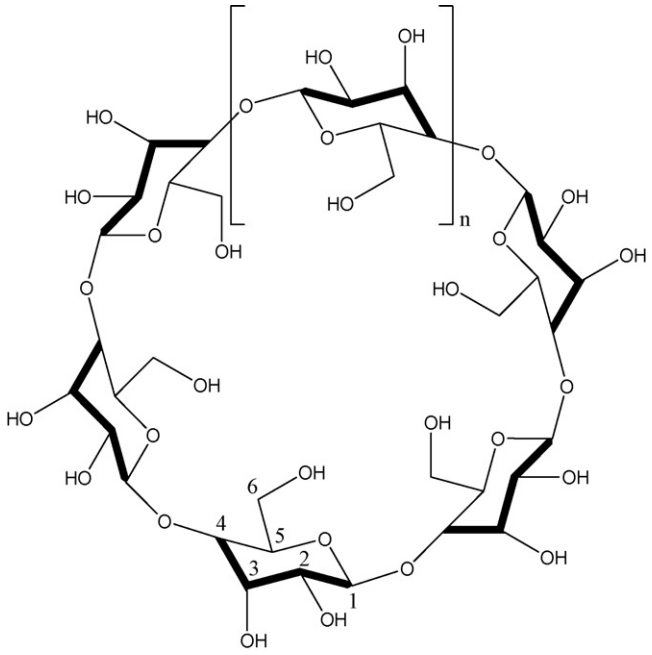


Fig. 2. α-1,4-linked D-glucopyranose units in cyclodextrins ($n=1$ αCD; $n=2$ βCD; $n=3$ γCD), showing the primary hydroxyl group in position C(6) and the two secondary ones in positions C(2) and C(3).

like methoxy functions, will convert the crystalline solids into amorphous mixture of CD isomers resulting in dramatic improvements in their aqueous solubility. CD derivatives of pharmaceutical interest 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 glucosyl-βCD (Loftsson and Brewster, 1996; Brewster and Loftsson, 2007).

Analysis of the crystal structure has shown that the cyclic structure of the CD-molecule is stabilized by intramolecular hydrogen bonds between the secondary OH-groups in position C(2) and C(3) of adjacent glucopyranose units (Bas and Rysanek, 1987). The secondary OH-groups assume defined orientations but the primary OH-groups are quite flexible and able to rotate in about the C(5)–C(6) bond (Fig. 2). Number of water molecules present in the stable hydrates of the CD lattices ranges from 6.4 for αCD to 14.2 for γCD (Table 1).

No covalent bonds are formed or broken during formation of guest/host inclusion complexes in aqueous solutions and guest molecules 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 complement of hydrogen bonds), van der Waals interactions, hydrophobic interactions, hydrogen bonds, electrostatic interactions, release of conformational and steric strain as well as charge-transfer interactions (Loftsson and Brewster, 1996; Liu and Guo, 2002). Physicochemical properties of free guest molecules are different from the complexes where the molecules are bound to the host (i.e. the CD) molecules. Likewise, the physicochemical properties of free host molecules are different from those in the complex. Any methodology that can be used to observe changes in additive physicochemical properties can, in theory, be utilized to determine the stoichiometry of the complexes formed and the numerical values of their stability constants (Hirayama and Uekama, 1987; Brewster and Loftsson, 1999; Hirose, 2001). These include changes in solubility, shifts in UV/vis absorbance, changes in chemical reactivity, as well as in fluorescence, NMR, drug retention (e.g. in liquid chromatography),

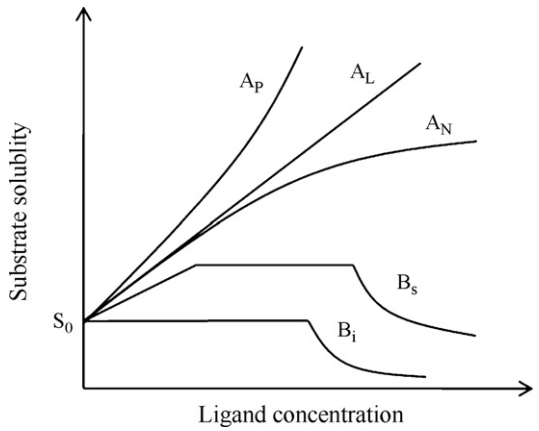


Fig. 3. Phase-solubility profiles and classification of complexes according to Higuchi and Connors (1965). S_0 is the intrinsic solubility of the substrate (i.e. the drug) in the aqueous complexation media, i.e. the solubility when no ligand (i.e. CD) is present.

pK_a values and potentiometric measurements, changes in chemical stability and effects on guest 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, changes in osmotic pressure, determinations of freezing point depression, vapor pressure lowering, viscosity measurements and calorimetric titrations. However, only few of these methods can be applied to obtain structural information on the guest/host complexes.

The Higuchi–Connor classification of the complexes is based on their phase-solubility diagrams, i.e. how the apparent solubility of a solute molecule (e.g. drug molecule) changes with increasing concentration of dissolved ligand (e.g. CD) due to enhanced aqueous solubility of the complex formed (Fig. 3) (Higuchi and Connors, 1965). In aqueous solutions A-type phase-solubility profiles are obtained when the solubility of the solute increases with increasing ligand concentration through formation of water-soluble complexes. When the complex is first order with respect to solute and first or higher order with respect to ligand then A_L-type phase-solubility profile is obtained. If the complex is first order with respect to the solute but second or higher order with respect to ligand then A_P-type phase-solubility profile is obtained. A_N-type phase-solubility profiles can be difficult to interpret but the negative deviation from linearity may be associated with ligand-induced changes in the dielectric constant of the aqueous complexation media, changes in complex solubility or aggregation of the ligand molecules. B-type phase-solubility profiles indicate formation of complexes with limited solubility in the aqueous complexation media. In general, the water-soluble CD derivatives form A-type phase-solubility profiles while the less soluble natural CDs frequently form B-type profiles. The phase-solubility profiles do not verify formation of inclusion complexes. They only describe how increasing CD concentration influences drug solubility. To distinguish between inclusion and other types of complexes experimental results from phase-solubility studies have to be supported by other experimental results from, for example, UV/vis, fluorescence and/or NMR studies (Loftsson et al., 2002a, 2004). The most common type of drug/CD complexes is the 1:1 drug/CD complex (D/CD) where one drug molecule (D) forms a complex with one CD molecule (CD):

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

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

intrinsic solubility (S_0) of the drug in the aqueous complexation media (i.e. drug solubility when no CD is present):

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (2)$$

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 αCD, βCD and γCD, respectively (Connors, 1995, 1997; Stella and Rajewski, 1997; Rao and Stella, 2003). For 1:1 drug/CD complexes the complexation efficiency (CE) can be calculated from the slope of the phase-solubility diagram (Loftsson et al., 2005):

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

CE is only a function of the slope and is not based on any particular substrate/ligand interaction. Thus, when selecting CD or complexation conditions during formulation work it can frequently be more convenient to compare the CEs than $K_{1:1}$ value.

CDs are also able to 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 (Gabelica et al., 2002). Likewise 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 (Correia et al., 2002) and some 1:2 and 2:2 drug/CD complexes have been shown to consist of a mixture of inclusion and non-inclusion complexes. This could explain why the values of the equilibrium constants (i.e. the K values) for the complex formation are sometimes concentration dependent and why their numerical values are frequently dependant on the method applied (Loftsson et al., 2002a, 2004). However, in dilute solutions inclusion-type guest/host CD complexes are probably more common than non-inclusion complexes. In aqueous solutions CDs are not only able to form inclusion and non-inclusion complexes, but also to form hydrogen bonds with neighboring CD molecules.

3. Self-aggregation of cyclodextrins

It has been known for some time that in aqueous solutions CD molecules have the tendency to self-associate to form aggregates, but the interest has gained momentum with recent advancements in analytical technologies (Table 2). To the best of our knowledge Miyajima and co-workers were first to suggest, based on their experimental results that CDs self-associate in aqueous solutions (Miyajima et al., 1983a, 1986). The activity coefficients, as well as viscosity and density dependencies on αCD and γCD concentrations, allowed the authors to warily assume that formation of CD dimers could possibly be the reason of the observed deviations from ideality. For the next decade physicochemical, pharmacokinetic and toxicological properties of CDs were intensively investigated. During development of CDs as pharmaceutical excipients and drug carriers many of their undesirable physicochemical properties were revealed and investigated, such as their limited aqueous solubility (Coleman et al., 1992) and spontaneous opalescence of aqueous CD solutions (Szente et al., 1998). The necessity to defy these 'undesirable' CD features arose and series of thoroughly planned studies were carried out by different teams using a wide variety of methods, assuming self-aggregation as the most probable explanation. Abundant data were obtained with light scattering (LS) methods, both dynamic and static, as they yield precious information on aggregate size and extent of aggregation process in a solution. Fig. 4 summarizes the results of these LS measurements conducted by different research groups (Coleman et al., 1992; González-Gaitano et al., 2002; Bonini et al., 2006; Wu et al., 2006a). The general observation is that the aggregates of the parent αCD, βCD and γCD tend to grow with increasing CD concentration. The largest aggregates are observed for βCD, which can be up to several micrometers in diameter. The anomalously low solubility of βCD is explained by the intensity of aggregate formation, which becomes notable at βCD concentrations above 3 mM (Bonini et al., 2006) and which is reversely proportional to hydration extent. It should be remembered that hydrogen bonds play important role in both processes, i.e. solubilization and aggregate formation. This explanation of low solubility of βCD is furthermore

Table 2
Some analytical methods that can be used to detect formation of CD aggregates.

Technique	Description	Advantages	Disadvantages	Examples
NMR (nuclear magnetic resonance)	Investigation of the electronic environment of an atom and its interaction with adjacent atoms	Very sensitive to changes in the molecular environment (e.g. forming of inclusion complexes)	Results can be difficult to interpret, especially when numerous atomic interactions are being investigated	Azaroual-Bellanger and Perly (1994)
LS (light scattering)	Determination of the hydrodynamic radius (R_h) of a particle by the light scattered from it	Direct observation of the particle size	Only R_h is obtained not the particle size and shape, difficult to find acceptable laser intensity that produces reliable results	González-Gaitano et al. (2002)
			Concentrated solutions produce signal noise; filtration or centrifugation of these solutions changes their composition	Bonini et al. (2006)
TEM (transmission electron microscopy)	Direct imagining of a sample by an electron beam	Image of the particles showing their sizes and shapes	Samples have to be solid and coated with gold or carbon	Bonini et al. (2006)
AFM (atomic force microscopy)	Mechanical scanning of particle surfaces	Direct observation of the particle size and shape	Cantilever must be able to scan the sample without scratching, particles must be fixed for scanning	Sakamoto et al. (2009)
Osmolality	Determination of changes in the freezing point depression or vapor pressure lowering due to formation of particles	Indirect observation of particle formation	No information about the particle shapes and sizes	Dagade et al. (2004)

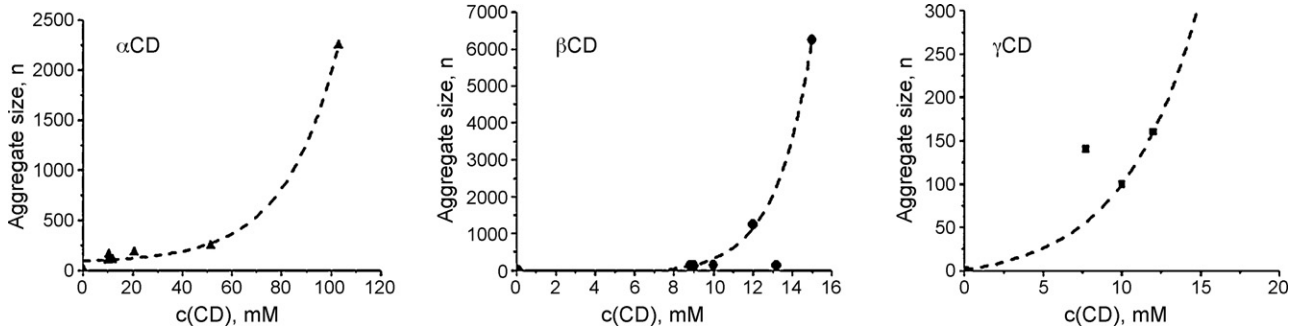


Fig. 4. An average size of native CD aggregates (*n* is the number of molecules) versus CD concentration observed by light scattering taken from literature (Coleman et al., 1992; González-Gaitano et al., 2002; Bonini et al., 2006; Wu et al., 2006a).

supported by several observations. For example, the substituted βCDs show significantly increased solubility with decreased or even abolished tendency of self-aggregation as in case of 2-methyl- and 3-methyl-βCD (Coleman et al., 1992). Both RMβCD and HPβCD show negligible aggregation at 12 mM while aggregate diameter does not exceed 70 molecules (González-Gaitano et al., 2002). Also, when the pH of aqueous CD solutions is increased to 12 or above, the OH groups of the CD molecule become ionized resulting in dissociation of the CD aggregates. Finally, chaotropic additives that break hydrogen bonds, such as urea or sodium chloride, cause notable depression of the self-association (Hausler and Muller-Goymann, 1993; Szente et al., 1998). These evidences clearly indicate that OH-groups of native CDs (Fig. 2) participate in self-aggregation rather than solvation. Since hydrogen bonds are known to be both saturable and direction dependent it is evident that intermolecular hydrogen binding of βCD molecules limits or prevents hydrogen bond formation with water that leads to solubility depression. The same explanation is valid for peculiarity of aqueous γCD solutions, which are well-known to become spontaneously turbid at γCD concentrations of 8 mM (1%, w/v) or above (Szente et al., 1998). A recent publication illustrates well the relationship between γCD aggregation and physicochemical properties such as diffusion coefficient, viscosity, activity coefficient and hydrodynamic radius (Ribeiro et al., 2008). It should be emphasized that formation of large aggregates does not necessarily indicate extensive aggregate formation. In pure aqueous CD solutions the fraction of molecules participating in aggregate formation is often very low. For example, the mass contribution of the αCD-aggregates in aqueous 12 mM αCD solution does not exceed 0.8% (González-Gaitano et al., 2002), that of βCD only 0.0011% in 10 mM in βCD solution (Wu et al., 2006b), and that of γCD only 0.02% in 12 mM γCD solution (Szente et al., 1998).

Another interesting feature of CD aggregates is their shape, which can be studied by a variety of microscopy techniques. This aspect was recently thoroughly reviewed (He et al., 2008). Along with spherical particles which can be observed in aqueous βCD and γCD solutions (Wu et al., 2006a,b) a wide spectrum of 'exotic' forms can be found. Especially wide variety of βCD aggregates have been reported by Bonini et al. (2006) using Cryo-TEM technique. In addition to spherical structures they found disks and large sheets consisting of welded fibers. Perhaps, the most natural arrangement of CDs in aggregates are long fibers (or rod, worm-like structures), that are organized similar to the channel type of CD crystal structure, i.e. stacks of molecules oriented in all possible ways: head-to-head, head-to-tail and tail-to-tail (Saenger et al., 1998). In addition, due to dynamic properties of a liquid solution medium and non-covalent binding abilities of CDs, such fibers can form higher-order congregations as mentioned previously (Table 3).

Alternatively self-aggregation can be studied by monitoring the colligative properties of CD solutions. This is based on the fact that formation oligomeric structures inevitably affect physicochemical properties such as vapor pressure, osmotic pressure, freezing point and boiling point. A classical way of studying solute aggregation is measurement solution's osmolality, either by monitoring changes in its vapor pressure or the freeze-point depression using pure solvent as a reference. The formation of aggregates decreases the number of particles in comparison to ideal solution, where the number of particles is equal to the number of non-electrolyte molecules, and aggregation is manifested as negative deviation of solution osmolality from its expected value. By mon-

Table 3
Self-assembly of the natural αCD, βCD and γCD.

	Aggregate shapes	Aggregate diameter at given concentration	Analytical method	Weight fraction of CD forming aggregates	References
αCD	Spherical coils (elongated nanostructures)	68 ± 20 nm (12 mM)	DLS	0.8% in 12 mM	Coleman et al. (1992); Polarz et al. (2001) and González-Gaitano et al. (2002)
βCD	Spherical disks, sheets, welded fibers, rod or worm-like structures	194 ± 10 nm (10 mM) 6 nm (3 mM)	DLS TEM	0.0011% in 10 mM	Coleman et al. (1992); Azaroual-Bellanger and Perly (1994); González-Gaitano et al. (2002); Bonini et al. (2006); Wu et al. (2006b) and Rossi et al. (2007)
γCD	Spherical	90 nm (3 mM) 174 ± 38 nm (12 mM) No aggregates 112 ± 37 nm (12 mM)	DLS DLS NMR DLS		Szente et al. (1998); González-Gaitano et al. (2002); Wu et al. (2006a)

itoring changes in osmolality as a function of CD concentration it is possible to estimate both the osmotic and activity coefficients of dissolved CD and determine its deviation from ideal CD solution. The method applied to obtain concentration dependencies of physicochemical properties, and the way the results are treated and interpreted, can be problematic. For example, Patel's team considered the negative concentration dependence of CD's apparent molar volume (φ_v) and positive concentration dependence of CD's activity coefficients (γ_2) as descriptors of solute–solute interaction, whereas Miyajima made the same conclusions based on opposite behavior of the noted magnitudes (Miyajima et al., 1983a; Dagade et al., 2004). The reason of such discrepancies can be due to differences in assumptions and simplifications applied. Different models often emphasize particular aspects of the method applied while underestimating or ignoring other aspects, or apply models that only relate to certain types of molecules such as the Robinson–Stokes model of solute–solvent equilibria for linear carbohydrates (Stokes and Robinson, 1966). Thermodynamic quantities do reflect overall changes in the solution such as reordering of solution structures and changes in hydration phenomena. Thus, all detailed conclusions regarding aggregate formation based on these thermodynamic studies have to be supported by other independent methods. However, Patel's team successfully applied different solution theories based on rigorous statistical mechanical notion like McMillan–Mayer and Kirkwood–Buff (Dagade et al., 2004; Terdale et al., 2006) to analyze solute–solute and solute–solvent interactions in αCD and βCD aqueous solutions. They assumed that both CD and water molecules were rigid spheres. Their results agreed with light scattering and microscopy studies and showed that solute–solute attractive interactions are considerably stronger than solute–solvent ones, which was attributed to hydrophobic interactions between βCD molecules. The presence of hydrophobic interactions between two or more αCD molecules in water has been suggested based on partial molar excess entropy increase with increase in temperature. This suggestion is further supported with the results of the noted theories application (Terdale et al., 2006).

While several different research groups, applying various experimental techniques, have shown that the parent CDs form nanosized aggregates in aqueous solutions, the situation is not as clear when it comes to the CD derivatives that can presently be found in marketed products. Some of these derivatives, such as the methylated or 2-hydroxypropylated CDs, demonstrate decreased ability to form aggregates compared to their parent CDs. This decreased ability can be explained in context of their hydrogen binding ability since replacement of the hydrogen atom in native CDs' hydroxyls by non-polar hydrocarbon groups leads to loss of their proton donating properties. In addition, random substitution of the OH-groups transforms the native CDs to a mixture of isomeric derivatives. Statistically, for example, there are about 130,000 possible heptakis(2-*O*-(hydroxypropyl)-β-cyclodextrin derivatives, and given that introduction of the 2-hydroxypropyl moiety also introduces an optically active center, the number of total isomers (i.e. geometrical and optical) is even much greater (Loftsson and Brewster, 1996). Furthermore, CD derivatives of pharmaceutical interest, such as HPβCD, SBEβCD and HPγCD, are mixtures of CD molecules with different degrees of substitution (i.e. different molecular weights). This fact decreases their ability to self-assemble to form crystalline aggregates. On the other hand, derivatives such as HPβCD contain additional centers of hydrogen bond formation (i.e. ether bridges), though less efficient than the hydroxyls, in comparison to native CD. The derivatization changes the physicochemical properties of the CDs resulting in, for example, considerable increase in their aqueous solubility. Hausler and Muller-Goymann applied several different methods (viscosimetry, polarized light microscopy, DSC, TEM, tensiometry) to study HPβCD behavior in aqueous solution. Anomalous increase of viscosity observed beyond 50% (w/w), as well as surface tension change at 0.5% (w/w), can be interpreted as either a consequence of strong hydration (accompanied with water structure reordering) or aggregation. The authors incline to the last variant, which was supported by TEM images showing 15–25 nm sized particles in 50% solution (Hausler and Muller-Goymann, 1993). Later, González-Gaitano et al. (2002) used dynamic light scattering to show that HPβCD has weak tendency to self-assemble to form aggregates yielding particles with hydrodynamic radius of 62 nm in aqueous 1.5% (w/w) solution. Thus, in spite of the presence of numerous hydrogen binding centers in HPβCD molecules, its aggregation ability is less pronounced than that of its parent analogue. Possibly the 2-hydroxypropyl moieties are sterically less favorable for solute-solute hydrogen bonding. It has been shown that the surface activity of amphiphilic CDs are directly proportional to their hydrophilicity, i.e. the more hydrophobic the derivative is the less thermodynamically stable nanoparticles are being formed (Mazzaglia et al., 2002). In addition, hydrophobic interactions of the substituents, rather than inclusion of hydrophobic chains, are thought to be the driving force for aggregation of hydrophobically modified CDs such as hydroxypropyl-phenylglycidyl-β-CD (Witte and Hoffmann, 1996). Thus, cohesion forces involving self-aggregation of the CD derivatives can be expected to be weaker than those of the native CDs and that the aggregates will collapse easily under unfavorable conditions like temperature increase, intensive shaking and sonication. For example, colligative properties of both HPβCD and HPγCD in solutions showed only small deviations from ideality at 37 °C, which was related to repulsion between CD molecules (Zannou et al., 2001). The reason of discrepant observations made by the various research groups can be due to instability of self-aggregates of the substituted CDs. Special attention must be paid to both the methods applied and the experimental conditions when experimental results from different sources are being compared.

Experimental data obtained by studying aqueous solutions of simple carbohydrates and oligosaccharides, such as of D-glucose, maltose and maltotriose, were used in an effort to elucidate the nature of interactions of CD molecules in aqueous solution (Miyajima et al., 1983b). The viscosity and apparent molar volume measurements showed that for oligosaccharides the solute–solute interactions appear and tend to intensify with increasing temperature, while for monomeric D-glucose these effects are practically absent. Similar conclusions were made when osmotic properties of glucose and sucrose solutions were investigated (Stigter, 1959). Monosaccharides are flexible and thus readily hydrated via hydrogen bonds. Formation α-1,4 ether bridges (e.g. formation of maltose and maltotriose) decrease the number of hydrogen binding sites and limits rotational freedom of molecular moieties. This decrease in hydrogen binding, together with increase of structural incompatibilities of the oligomers with water, leads to hydration depression and creates prerequisites for self-aggregation. In case of CDs the geometric factors are even more profound. Furthermore, not only CDs are believed to be able to form aggregates, but also their guest/host complexes.

4. Aggregation of cyclodextrin complexes

Complexation changes the physicochemical properties of CDs, including their ability to form aggregates. Formation of inclusion complex with lipophilic guest molecules can convert a CD molecule from a simple oligosaccharide to a surfactant-like molecule capable to form micellar-type aggregates. Also, poorly soluble guests can participate in formation of guest/CD co-aggregates where the molecules are kept together through non-inclusion complex formations. This makes the aggregation of CD complexes more diverse

Table 4
Overview on aggregates of CD complexes.

CD	Guest molecule	Complex type	Observing method	Reference
βCD	Trans-β-carotene	Micelle-like	LS/NMR	Mele et al. (1998)
γCD	Trans-β-carotene	Micelle-like	LS/NMR	Mele et al. (1998)
	Hydrocortisone		Permeation	Jansook et al. (2010)
HPβCD	Non-steroidal drugs + Na-salts	Micelle-like		Magnusdottir et al. (2002)
	Hydrocortisone	Micelle-like	Permeation	Loftsson et al. (2002a)
	Cholesterol	Micelle-like		Loftsson et al. (2002b)
	Diflunisal	Micelle-like		Loftsson et al. (2002b)
HPγCD	Hydrocortisone		Permeation	Jansook et al. (2010)
Parent CDs	Surfactant	Micelle-like	Job plots	Liptaj et al. (1995)
Hydrophobic CDs	Surface-active guest	Micelle-like		Auzely-Velty et al. (2001)
CDs	Fatty acids	Micelle-like	Microscopy	Skiba et al. (1996) and Bochot et al. (2007)

leading new dimensions and designs of drug delivery systems (Table 4).

The aggregation of CD complexes can be driven by guests, especially by those guests that tend to self-aggregate themselves. In particular, native CDs demonstrate more pronounced aggregation when they coexist in solution with hydrophobic guests such as trans-β-carotene. Light scattering measurements have shown that even at low concentrations (less than 0.1%, w/v) both βCD and γCD form relatively large aggregates when trans-β-carotene was present (Mele et al., 1998). Additional NMR studies yielded chemical shifts pattern indicating presence of other interaction mechanisms along with inclusion complexation. The authors suggested a micelle-like structure of aggregates with hydrophobic guest in the core and hydrophilic palisade of host CDs. In another study aggregation governed by surface-active guest was observed by Liptaj et al. (1995) that, on the basis of asymmetrical Job's plots, indicated formation of pre-micellization surfactant/βCD complexes. Due to different physicochemical properties of the formed complex the mechanism of aggregate formation can be completely different from the one of self-aggregation of native CDs and their derivatives in pure aqueous solutions. Still another type of CD aggregation is micellization of hydrophobically modified CDs, where the micelle core is formed by hydrophobic moieties of CD itself. Such aggregate formations can be influenced by guest nature. For example, a surface-active guest can both form inclusion complex and participate in mixed micelle formation (Auzely-Velty et al., 2001). This type of complex association can offer new possibilities in drug delivery since the shape of aggregates formed preserves their ability to form inclusion complexes with hydrophobic drugs.

Other evidence of complex aggregation does exist. For example, during phase-solubility studies of sodium salts of some non-steroidal drugs in aqueous HPβCD solution a diagram with slope greater than unity was obtained indicating that 2:1 or 3:1 (drug/HPβCD) complexes were being formed, while Job's plots based on both UV and NMR measurements indicated that the stoichiometry of the drug/HPβCD complexes were 1:1 (Magnusdottir et al., 2002). The micelle formation was contemplated due to drug ionization, but the aqueous complexation media were found to be free of surfactant-like properties. Further analysis of solutions containing two or more different drugs led to the conclusion that non-inclusion interactions participated in the drug/HPβCD complex formation. This non-inclusion phenomenon of complex formation indicates that a drug molecule can serve as an aggregation promoter. Other studies involving drug permeation from aqueous solutions containing increasing concentration of drug/CD complexes through semipermeable cellophane membranes with molecular weight cut off (MWCO) from 500 to 100,000 showed that 1:1 hydrocortisone/HPβCD complexes give negative deviation from Fick's first law (Fig. 5) which states that the flux will increase proportional to increasing drug concentration in the donor phase (Loftsson et al., 2002b). TEM studies of hydrocortisone/HPγCD and hydrocortisone/γCD complex solutions confirmed that complexes of both native CDs and their derivatives form nanosized aggregates (Jansook et al., 2010). Fig. 6 shows hydrocortisone/HPγCD and hydrocortisone/γCD complex aggregates. The diameter of the homogeneous aggregates formed in aqueous hydrocortisone/γCD solutions was approximately 20 nm while the aggregates formed in hydrocortisone/HPγCD solutions were polydisperse with diameter ranging from 10 to 80 nm. Nanoparticles of this size contain large number of CD and hydrocortisone molecules that combined exceed the MWCO of the membranes giving rise to the previously mentioned negative deviation from Fick's first law during drug permeation studies (Fig. 5) (Loftsson et al., 2004). Our permeation studies have indicated that at HPγCD concentration of about 1% (w/v) or lower the relative mass contribution of these aggregates is less than 0.01% but it gradually increases with increasing HPγCD concentration until at about 5–10% (w/v) when all increase in dissolved drug/cyclodextrin complexes is in the form of cyclodextrin

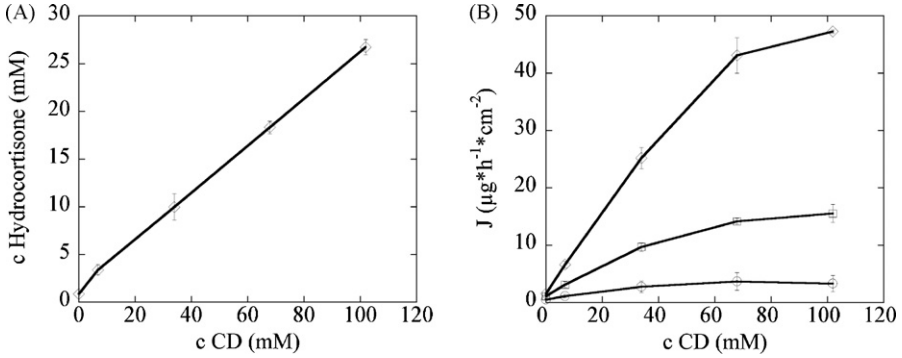


Fig. 5. A_L-type phase-solubility profile of hydrocortisone in aqueous HPβCD solution (A) and hydrocortisone flux-HPβCD concentration profiles (B) through 100,000 (◊) 50,000 (□) and 15,000 (○) MWCO membranes (unpublished data).

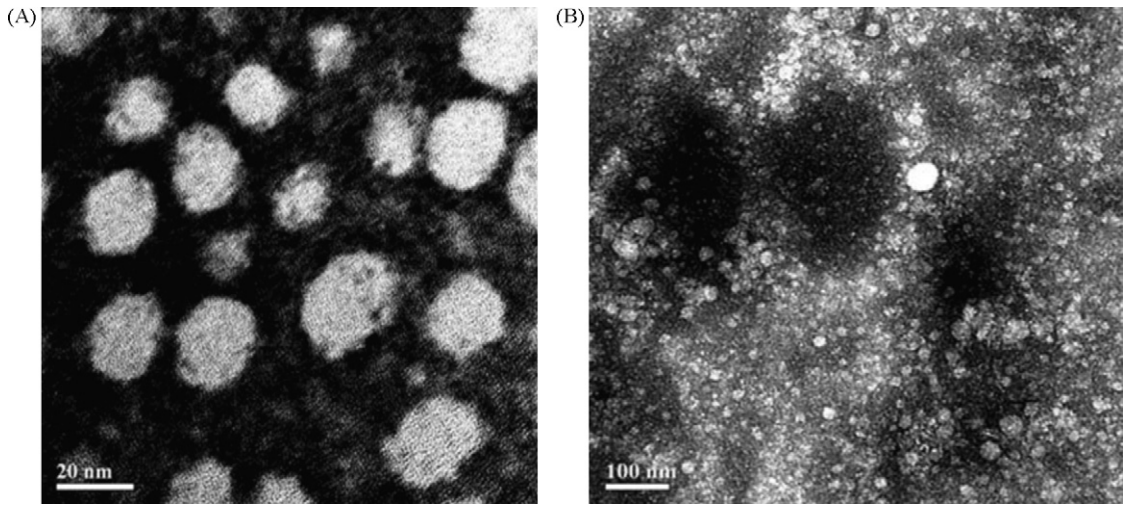


Fig. 6. Transmission electron microscopic (TEM) images of saturated solution of hydrocortisone in 10% (w/v) cyclodextrin solutions: aggregated hydrocortisone/γCD complexes (A); hydrocortisone/HPγCD complexes (B) (unpublished data).

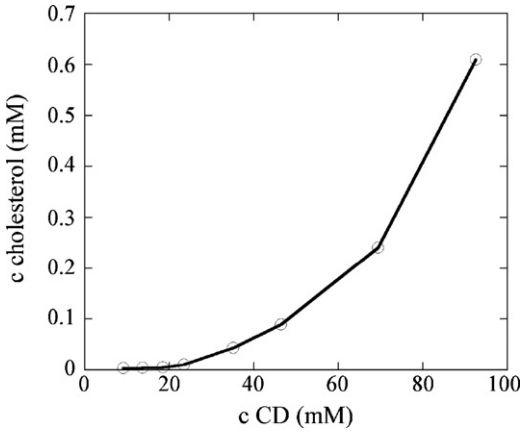


Fig. 7. A_P-type phase-solubility diagram of cholesterol in aqueous SBEβCD solution (based on Loftsson et al., 2002a).

aggregates (Jansook et al., 2010). Similar results have been obtained for other CDs, e.g. HPβCD and SBEβCD, and other drugs (unpublished results). Formation complex aggregates can also explain why phase-solubility diagrams of cholesterol in aqueous cyclodextrin solutions show positive deviation from linearity (i.e. are of A_P type) (Fig. 7) (Loftsson et al., 2002a) and why cholesterol saturated HPβCD solutions are able to dissolve 30% more amphotericin B than comparable pure aqueous HPβCD solutions. Similarly alprazolam has about 50% higher solubility in diflunisal saturated HPβCD solutions than in pure aqueous HPβCD solutions (Loftsson et al., 2002a). Such observations indicate that the cholesterol/HPβCD and diflunisal/HPβCD complexes are forming micelle-like structures that are able to solve poorly soluble drugs by micellar fashion. Such micelle-like structures are also formed in aqueous solutions by fatty acid/CD complexes but these have diameters close to 1 mm (Skiba et al., 1996; Bochot et al., 2007).

5. The effect of aggregate formation on CD solubilization

Certain pharmaceutical excipients are known to enhance CD solubilization of drugs. The examples given in Table 5 suggest formation of complex aggregates and non-inclusion interactions between these aggregates and other excipients present in the complexation media. Molecular modeling studies have suggested that malic acid acts as go-between econazole/αCD inclusion complex aggregates reinforcing nanostructures via specific (hydrogen bond and salt bridging) interactions with both components (Faucci et al., 2000). Similarly significant improvement of the complexation efficiency is observed when small amount of water-soluble polymers is present in the aqueous complexation media (Loftsson et al., 1994, 2002a, 2003). This observation is analogous to the effects these same polymers have on the solubilizing effects of micelles (Brackman and Engberts, 1993). Thus, it is likely that the

Table 5
Methods that can be applied to enhance CD solubilization of drugs.

Effect	Consequences	References
The acid/base ternary complexes	Certain organic hydroxy acids and certain organic bases are able to enhance the complexation efficiency by formation of ternary drug/CD/acid or base complexes	Tinwalla et al. (1993); Selva et al. (1998); Fenyvesi et al. (1999); Redenti et al. (2000) and Mura et al. (2003)
Polymer complexes	Water-soluble polymers form a ternary complex with drug/CD complexes increasing the observed stability constant of the drug/CD complex. This observed increase in the value of the constant increases the complexation efficiency	Loftsson and Másson (2004)
Ion pairing	Ion pairing of positively charged compounds with negatively charged CDs enhances the complexation efficiency	Zia et al. (2001)
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 solubilization of CD aggregates by adding both polymers and cations or anions to the aqueous complexation medium	Granero et al. (2003); Loftsson et al. (2003) and Loftsson and Másson (2004)

polymers change and perhaps stabilize the drug/CD complex aggregates through formation of CD–polymer hydrogen bonds (Ribeiro et al., 2003). In addition, the polymers are known to increase aqueous solubility of βCD and drug/βCD complexes (Loftsson and Friðriksdóttir, 1998).

6. Conclusions and future directions

In aqueous solutions CDs self-assemble to form nanosized aggregates. The aggregate formation is concentration dependent increasing with increasing CD concentration. In the case of the natural αCD, βCD and γCD the fraction of CD molecules forming aggregates is in most cases less than 1%, and frequently less than 0.1%, in pure aqueous 1% (w/v) CD solutions while the hydrophilic CD derivatives, e.g. the hydroxypropylated and sulfobutylether derivatives, appear to have even less tendency to self-assemble to form aggregates at these low concentrations. Then the fraction of CD molecules forming aggregates gradually increases with increasing CD concentration. Formation of guest/host inclusion complexes can increase significantly the tendency of CD molecules to form aggregates. Some CD complexes have surfactant-type structure that lends CD molecules the ability to form micellar-type aggregates that as such possess additional solubilizing properties. The aggregate phenomenon has to be considered during investigation of CD containing solutions. This is especially critical during drug formulation studies where concentrations of both drug and CD are relatively high. Following are few points that need to be considered:

- Aggregate formation is enhanced when relatively lipophilic drug molecules form inclusion complexes with hydrophilic CD molecules. It is thought that such complexes have a tendency to form micellar-type aggregates.
- Inclusion complexes predominate in relatively dilute aqueous CD solutions. Thus, characterization of drug/CD inclusion complexes should be performed in dilute solutions. However, studies of dilute aqueous CD solutions, e.g. by NMR or UV, cannot be used to explain phenomenon observed in more concentrated CD solutions without further validation.
- The presence and stoichiometry of CD inclusion complexes cannot be determined by phase-solubility studies. A Higuchi's A_L-type phase-solubility diagram does not necessarily indicate formation of 1:1 drug/CD complexes and A_P-type diagram does not necessarily indicate formation of 1:2 drug/CD complexes. Phase-solubility studies are carried out in aqueous drug saturated, and sometimes CD saturated, solutions under conditions that promote aggregate formation.
- Frequently inclusion complexes as well as various types and sizes of aggregates are simultaneously present in aqueous complexation media. Furthermore, the aggregate formation is concentration dependent. Thus, apparent stability constants of drug/CD complexes can be both method and concentration dependent.
- Excipients that stabilize and/or solubilize nanoparticles, such as water-soluble polymers, low-molecular weight organic acids and surfactants, can improve the solubilizing effect of CD.
- Since pharmaceutical excipients can influence the aggregate formation the amount of CD needed to produce certain effect, e.g. drug solubilization or stabilization, should be determined by in a complexation media that closely resembles the final drug formulation.
- Formation of CD aggregates is a revisable process and will, in general, not affect quantitative drug determination by HPLC or influence drug bioavailability from CD containing formulations.

References

Auzely-Velty, R., Pean, C., Djeddaini-Pilard, F., Zemb, T., Perly, B., 2001. Micellization of hydrophobically modified cyclodextrins: 2. Inclusion of guest molecules. Langmuir 17, 504–510.

Azaroual-Bellanger, N., Perly, B., 1994. Investigation of the dynamics of cyclodextrins and their inclusion complexes in water by deuterium NMR. Magn. Reson. Chem. 32, 8–11.

Bas, G.L., Rysanek, N., 1987. Structural aspects of cyclodextrins. In: Duchêne, D. (Ed.), Cyclodextrins and Their Industrial uses. Editions de Santé, Paris.

Bochot, A., Trichard, L., Le Bas, G., Alphandary, H., Grossiord, J.L., Duchene, D., Fattal, E., 2007. Alpha-cyclodextrin/oil beads: an innovative self-assembling system. Int. J. Pharm. 339, 121–129.

Bonini, M., Rossi, S., Karlsson, G., Almgren, M., Lo Nostro, P., Baglioni, P., 2006. Self-assembly of β-cyclodextrin in water. Part 1: Cryo-TEM and dynamic and static light scattering. Langmuir 22, 1478–1484.

Brackman, J.C., Engberts, J.B.F.N., 1993. Polymer–micelle interactions: physical organic aspects. Chem. Soc. Rev., 85–92.

Brewster, M.E., Loftsson, T., 1999. Complexation—use of cyclodextrins to improve pharmaceutical properties of intramuscular formulations. In: Gupta, P.K., Brazeau, G.A. (Eds.), Injectable Drug Development. Techniques to Reduce pain and Irritation. Interpharm Press, Denver.

Brewster, M.E., Loftsson, T., 2007. Cyclodextrins as pharmaceutical solubilizers. Adv. Drug Deliv. Rev. 59, 645–666.

Coleman, A.W., Nicolis, I., Keller, N., Dalbiez, J.P., 1992. Aggregation of cyclodextrins: an explanation of the abnormal solubility of β-cyclodextrin. J. Incl. Phenom. Macrocycl. Chem. 13, 139–143.

Connors, K.A., 1995. Population characteristics of cyclodextrin complex stabilities in aqueous solution. J. Pharm. Sci. 84, 843–848.

Connors, K.A., 1997. The stability of cyclodextrin complexes in solution. Chem. Rev. 97, 1325–1357.

Correia, I., Bezzenine, N., Ronzani, N., Platzer, N., Beloeil, J.-C., Doan, B.-T., 2002. Study of inclusion complexes of acridine with β- and (2,6-di-*O*-methyl)-β-cyclodextrin by use of solubility diagrams and NMR spectroscopy. J. Phys. Org. Chem. 15, 647–659.

Dagade, D.H., Kolhapurkar, R.R., Patil, K.J., 2004. Studies of osmotic coefficients and volumetric behaviour on aqueous solutions of beta-cyclodextrin at 298.15 K. Ind. J. Chem. Sec. A 43, 2073–2080.

Davis, M.E., Brewster, M.E., 2004. Cyclodextrin-based pharmaceutics: past, present and future. Nat. Rev. 3, 1023–1035.

De Jong, W.H., Borm, P.J.A., 2008. Drug delivery and nanoparticles: applications and hazards. Int. J. Nanomed. 3, 133–149.

Dodziuk, H. (Ed.), 2006. Cyclodextrins and Their Complexes. Wiley-VCH Verlag, Weinheim.

Faucci, M.T., Melani, F., Mura, P., 2000. H-1-NMR and molecular modelling techniques for the investigation of the inclusion complex of econazole with alpha-cyclodextrin in the presence of malic acid. J. Pharm. Biomed. Anal. 23, 25–31.

Fenyvesi, E., Vikmon, M., Szeman, J., Redenti, E., Delcanale, M., Ventura, P., Szejtli, J., 1999. Interaction of hydoxy acids with β-cyclodextrin. J. Incl. Phenom. Macroc. Chem. 33, 339–344.

Frömming, K.H., Szejtli, J., 1994. Cyclodextrins in Pharmacy. Kluwer Academic Publishers, Dordrecht.

Gabelica, V., Galic, N., De Pauw, E., 2002. On the specificity of cyclodextrin complexes detected by electrospray mass spectrometry. J. Am. Soc. Mass Spectrom. 13, 946–953.

González-Gaitano, G., Rodríguez, P., Isasi, J.R., Fuentes, M., Tardajos, G., Sánchez, M., 2002. The aggregation of cyclodextrins as studied by photon correlation spectroscopy. J. Incl. Phenom. Macrocycl. Chem. 44, 101–105.

Granero, G., Granero, C., Longhi, M., 2003. The effect of pH and triethanolamine on sulfisoxazole complexation with hydroxypropyl-β-cyclodextrin. Eur. J. Pharm. Sci. 20, 285–293.

Hausler, O., Muller-Goymann, C.C., 1993. Properties and structure of aqueous-solutions of hydroxypropyl-beta-cyclodextrin. Starch–Starke 45, 183–187.

He, W., Fu, P., Shen, X.H., Gao, H.C., 2008. Cyclodextrin-based aggregates and characterization by microscopy. Micron 39, 495–516.

Higuchi, T., Connors, K.A., 1965. Phase-solubility techniques. Adv. Anal. Chem. Instrum. 4, 117–212.

Hirayama, F., Uekama, K., 1987. Methods of investigating and preparing inclusion compounds. In: Duchêne, D. (Ed.), Cyclodextrins and Their Industrial uses. Editions de Santé, Paris.

Hirose, K., 2001. A practical guide for the determination of binding constants. J. Incl. Phenom. Macrocycl. Chem. 39, 193–209.

Jansook, P., Kurkov, S.V., Loftsson, T., 2010. Cyclodextrins as solubilizers: formation of complex aggregates. J. Pharm. Sci. 99, 719–729.

Kreuter, J. (Ed.), 1994. Colloidal Drug Delivery Systems. Dekker, New York.

Liptaj, T., Pronayova, N., Karlova, K., 1995. NMR-study of the inclusion complex of cationic surfactant N,N′-bis [4-(4-tert-butylphenoxy)butyldimethyl]-1,6-hexanediammonium dibromide with beta-cyclodextrin. Pharmazie 50, 744–747.

Liu, L., Guo, Q.-X., 2002. The driving forces in the inclusion complexation of cyclodextrins. J. Incl. Phenom. Macrocycl. Chem. 42, 1–14.

Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.

Loftsson, T., Friðriksdóttir, H., 1998. The effect of water-soluble polymers on the aqueous solubility and complexing abilities of β-cyclodextrin. Int. J. Pharm. 163, 115–121.
Loftsson, T., Fridriksdottir, H., Sigurdardottir, A.M., Ueda, H., 1994. The effect of water-soluble polymers on drug–cyclodextrin complexation. Int. J. Pharm. 110, 169–177.
Loftsson, T., Magnúsdóttir, A., Másson, M., Sigurjónsdóttir, J.F., 2002a. Self-association and cyclodextrin solubilization of drugs. J. Pharm. Sci. 91, 2307–2316.
Loftsson, T., Másson, M., Sigurdsson, H.H., 2002b. Cyclodextrins and drug permeability through semi-permeable cellophane membranes. Int. J. Pharm. 232, 35–43.
Loftsson, T., Matthíasson, K., Másson, M., 2003. The effects of organic salts on the cyclodextrin solubilization of drugs. Int. J. Pharm. 262, 101–107.
Loftsson, T., Másson, M., 2004. The effects of water-soluble polymers on cyclodextrins and cyclodextrin solubilization of drugs. J. Drug Del. Sci. Tech. 14, 35–43.
Loftsson, T., Másson, M., Brewster, M.E., 2004. Self-association of cyclodextrins and cyclodextrin complexes. J. Pharm. Sci. 93, 1091–1099.
Loftsson, T., Hreinsdóttir, D., Másson, M., 2005. Evaluation of cyclodextrin solubilization of drugs. Int. J. Pharm. 302, 18–28.
Magnusdottir, A., Másson, M., Loftsson, T., 2002. Self association and cyclodextrin solubilization of NSAIDs. J. Incl. Phenom. Macrocycl. Chem. 44, 213–218.
Mazzaglia, A., Scolaro, L.M., Darcy, R., Donohue, R., Ravoo, B.J., 2002. Entangled porphyrins in cyclodextrin vesicles. J. Incl. Phenom. Macrocycl. Chem. 44, 127–132.
Mele, A., Mendichi, R., Selva, A., 1998. Non-covalent associations of cyclomaltooligosaccharides (cyclodextrins) with *trans*-β-carotene in water: evidence for the formation of large aggregates by light scattering and NMR spectroscopy. Carbohydr. Res. 310, 261–267.
Miyajima, K., Sawada, M., Nakagaki, M., 1983a. Viscosity B-coefficients, apparent molar volumes, and activity-coefficients for alpha-cyclodextrin and gamma-cyclodextrin in aqueous-solutions. Bull. Chem. Soc. Jpn. 56, 3556–3560.
Miyajima, K., Sawada, M., Nakagaki, M., 1983b. Studies on aqueous-solutions of saccharides. 2. Viscosity B-coefficients, apparent molar volumes, and activity-coefficients of D-glucose, maltose and maltotriose in aqueous-solutions. Bull. Chem. Soc. Jpn. 56, 1954–1957.
Miyajima, K., Mukai, T., Nakagaki, M., Otagiri, M., Uekama, K., 1986. Activity-coefficients of dimethyl-beta-cyclodextrin in aqueous-solutions. Bull. Chem. Soc. Jpn. 59, 643–644.
Mura, P., Maestrelli, F., Cirri, M., 2003. Ternary systems of naproxen with hydroxypropyl-β-cyclodextrin and aminoacids. Int. J. Pharm. 260, 293–302.
Patel, T.R., Harding, S.E., Ebringerova, A., Deszczynski, M., Hromadkova, Z., Togola, A., Paulsen, B.S., Morris, G.A., Rowe, A.J., 2007. Weak self-association in a carbohydrate system. Biophys. J. 93, 741–749.
Polarz, S., Smarsly, B., Bronstein, L., Antonietti, M., 2001. From cyclodextrin assemblies to porous materials by silica templating. Angew. Chem. Int. Ed. 40, 4417–4421.
Rao, V.M., Stella, V.J., 2003. When can cyclodextrins be considered for solubilizing purposes? J. Pharm. Sci. 92, 927–932.
Redenti, E., Szente, L., Szejtli, J., 2000. Drug/cyclodextrin/hydroxy acid multicomponent systems. Properties and pharmaceutical applications. J. Pharm. Sci. 89, 1–8.
Ribeiro, L., Ferreira, D., Veiga, F., 2003. Physicochemical investigation of the effects of water-soluble polymers on vinpocetine complexation with β-cyclodextrin and its sulfobutyl ether derivative in solution and solid state. Eur. J. Pharm. Sci. 20, 253–266.
Ribeiro, A.C.F., Santos, C., Valente, A.J.M., Ascenso, O.S., Lobo, V.M.M., Burrows, H.D., Cabral, A., Veiga, F.J.B., Teijeiro, C., Esteso, M.A., 2008. Some transport properties of gamma-cyclodextrin aqueous solutions at (298.15 and 310.15) K. J. Chem. Eng. Data 53, 755–759.
Rossi, S., Bonini, M., Nostro, P.L., Baglioni, P., 2007. Self-assembly of β-cyclodextrin in water: electron spin resonance. Langmuir 23, 10959–10967.
Sabadini, E., Cosgrove, T., Egídio, F.C., 2006. Solubility of cyclomaltooligosaccharides (cyclodextrins) in H_2O and D_2O: a comparative study. Carbohydr. Res. 341, 270–274.
Saenger, W., Jacob, J., Gessler, K., Steiner, T., Hoffmann, D., Sanbe, H., Koizumi, K., Smith, S.M., Takaha, T., 1998. Structures of the common cyclodextrins and their larger analogues—beyond the doughnut. Chem. Rev. 98, 1787–1802.
Sakamoto, K., Takashima, Y., Tamaguchi, H., Harada, A., 2009. Supramolecular assemblies of oligothiophene derivatives bearing beta-cyclodextrin. Synth. Met. 159, 977–981.
Sanvicens, N., Marco, M.P., 2008. Multifunctional nanoparticles—properties and prospects for their use in human medicine. Trends Biotechnol.
Selva, A., Redenti, E., Ventura, P., Zanol, M., Casetta, B., 1998. Study of β-cyclodextrin-ketakonazole-tartaric acid multicomponent non-covalent association by positive and netagive ionspray mass spectrometry. J. Mass Sepectrom. 33, 729–734.
Skiba, M., Wouessidjewe, D., Puisieux, F., Duchene, D., Gulik, A., 1996. Characterization of amphiphilic beta-cyclodextrin nanospheres. Int. J. Pharm. 142, 121–124.
Stella, V.J., Rajewski, R.A., 1997. Cyclodextrins: their future in drug formulation and delivery. Pharm. Res. 14, 556–567.
Stigter, D., 1959. Interactions in aqueous solutions. II. Osmotic pressure and osmotic coefficient of sucrose and glucose solutions. J. Phys. Chem. 64, 118–124.
Stokes, R.H., Robinson, R.A., 1966. Interactions in aqueous nonelectrolyte solutions. Solute–solvent equilibria. J. Phys. Chem. 70, 2126–2131.
Szente, L., Szejtli, J., Kis, G.L., 1998. Spontaneous opalescence of aqueous γ-cyclodextrin solutions: complex formation or self-aggregation. J. Pharm Sci. 87, 778–781.
Terdale, S.S., Dagade, D.H., Patil, K.J., 2006. Thermodynamic studies of molecular interactions in aqueous alpha-cyclodextrin solutions: application of McMillan–Mayer and Kirkwood–Buff theories. J. Phys. Chem. B 110, 18583–18593.
Tinwalla, A.Y., Hoesterey, B.L., Xiang, T.-X., Lim, K., Anderson, B.D., 1993. Solubilization of thiazolobenzimidazole using a combination of pH adjustment and complexation with 2-hydroxypropyl-β-cyclodextrin. Pharm. Res. 10, 1136–1143.
Trichard, L., Duchêne, D., Bochot, A., 2006. Cyclodextrins in dispersed systems. In: Dodziuk, H. (Ed.), Cyclodextrins and Their Complexes: Chemistry, Analytical Methods, Applications. Wiley-VCH, Weinheim.
Witte, F., Hoffmann, H., 1996. Aggregation behavior of hydrophobically modified beta-cyclodextrins in aqueous solution. J. Incl. Phenom. Mol. Rec. Chem. 25, 25–28.
Wu, A., Shen, X., He, Y., 2006a. Investigation of γ-cyclodextrin nanotube induced by *N*,*N*′-diphenylbenzidine molecule. J. Colloid Interf. Sci. 297, 525–533.
Wu, A.H., Shen, X.H., He, Y.K., 2006b. Micrometer-sized rodlike structure formed by the secondary assembly of cyclodextrin nanotube. J. Colloid Interf. Sci. 302, 87–94.
Zannou, E.A., Streng, W.H., Stella, V.J., 2001. Osmotic properties of sulfobutylether and hydroxypropyl cyclodextrins. Pharm. Res. 18, 1226–1231.
Zia, V., Rajewski, R.A., Stella, V.J., 2001. Effect of cyclodextrin charge on complexation of neutral and charged substrates: comparison of (SBE)7M-β-CD to HP-β-CD. Pharm. Res. 18, 667–673.