REVIEW

Faculty of Pharmacy[1], Siam University, Bangkok; College of Pharmacy[2], Rangsit University, Pathum Thani; Faculty of Pharmaceutical Sciences[3], Chulalongkorn University, Bangkok, Thailand; Faculty of Pharmaceutical Sciences[4], University of Iceland, Reykjavik, Iceland

Self-assembly of cyclodextrin complexes: detection, obstacles and benefits

C. Muankaew[1,#], P. Saokham[2,#], P. Jansook[3,#], T. Loftsson[4,*]

Received March 9, 2020, accepted April 17, 2020

**Corresponding author: Thorsteinn Loftsson, Faculty of Pharmaceutical Sciences, University of Iceland, Hofsvallagata 53, IS-107 Reykjavik, Iceland*
thorstlo@hi.is
[#]These authors contributed equally to this paper.

Pharmazie 75: 307-312 (2020) *doi: 10.1691/ph.2020.0405*

Cyclodextrins (CDs) are cyclic oligosaccharides that form water-soluble inclusion complexes of lipophilic molecules. They are commonly used as pharmaceutical excipients. Recently it has been observed that CDs and CD complexes self-assemble in aqueous solutions to form transient clusters, nanoparticles and small microparticles. The critical aggregation concentration (cac) of the natural αCD, βCD and γCD in pure aqueous solutions is about 25, 8 and 9 mg/ml, respectively. The cac of 2-hydroxypropyl-β-cyclodextrin (HPβCD), that consists of a mixture of isomers, in pure aqueous solutions is significantly higher or about 118 mg/ml. Formation of guest/CD complexes can increase or decrease the cac value. Due to the transient nature of the CD clusters and nanoparticles they can be difficult to detect and their presence is frequently ignored. However, formation of such particulate matter in aqueous CD solutions can lead to erroneous analytical results and product rejections during drug manufacturing. On the other hand, they have also given rise to formation of novel drug delivery systems with exceptional properties.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides, formed by α-1,4 linked glucose units, with a somewhat lipophilic central cavity and hydrophilic outer surface (Table 1, Fig. 1). They are frequently referred to as enabling excipients that can, for example, increase aqueous solubility of poorly soluble compounds, increase both chemical and physical stability of drug products, and increase drug bioavailability. In other words, CDs can enable pharmaceutical formulations of biologically active compounds during preparation of marketable drug products. Recently it has been shown that CDs can self-assemble to form aggregates in aqueous solutions (Loftsson et al. 2019; Zagami et al. 2019). Here the obstacles and advantages that formation of such CD aggregates can generate in drug delivery are discussed.

Three natural CDs (i.e. the unsubstituted or parent CDs) and four CD derivatives are currently used in marketed drug products as enabling excipients (Table 1). The natural CDs (i.e. αCD, βCD and γCD) and their complexes have somewhat limited solubility in water. Random substitution of their external hydroxy functions (i.e. formation of HPβCD, SBEβCD, RMβCD and HPγCD) increases their solubility and that of their complexes. Thus, the natural CDs are mainly used in solid dosage forms while the CD derivatives are used in drug preparations that are aqueous solutions.

In general, topically or orally administered drugs have to permeate some lipophilic biomembranes to enter the body and produce desired therapeutic effects, and only dissolved drug molecules are able to permeate the membranes. Most often biomembranes such as mucosa contain aqueous exterior (e.g., mucus) and in general drug permeate the membranes *via* passive diffusion where the gradient of dissolved drug molecules is the driving force. The permeation barrier consists of not only the lipophilic membrane but also the aqueous exterior. For drug permeation through the barrier the drug must be somewhat water-soluble to dissolve in the aqueous exterior and, at the same time, somewhat lipophilic to be

Table 1: Cyclodextrins (CDs) currently used in marketed pharmaceutical products

Cyclodextrin	Number of glucose units [1]	MS [2]	MW [3] (g/mol)	S_{water} [4] (mg/ml)	Pharmaceutical applications in marketed products
α-Cyclodextrin (αCD)	6	-	972.8	145	Solid dosage forms and parenteral solutions.
β-Cyclodextrin (βCD)	7	-	1135	18.5	Solid dosage forms (e.g., tablets and suppositories).
2-Hydroxypropyl-βCD (HPβCD)	7	0.65	1400	> 600	Aqueous solutions (e.g., eye drops and IV solutions).
Sulfobutylether βCD sodium salt (SBEβCD)	7	0.9	2163	> 500	Aqueous solutions (e.g., IV and IM solutions).
Randomly methylated βCD (RMβCD)	7	1.8	1312	> 600	Aqueous solutions (e.g., eye drops and nasal spray).
γ-Cyclodextrin (γCD)	8	-	1297	232	Aqueous IV solution.
2-Hydroxypropyl-γCD (HPγCD)	8	0.6	1576	> 500	Aqueous solutions (e.g., eye drops and IV solution).

[1] Number of glucose units forming the cyclic CD molecule.
[2] The molar degree of substitution (MS) is defined as the average number of substituents that have reacted with one glucopyranose repeat unit.
[3] Molecular weight (MW) of the unhydrated CD.
[4] S_{water} is the solubility in pure water at room temperature (Dodziuk 2006, Szejtli 1988)

able to permeate the membrane. Chemical penetration enhancers, such as ethanol, dimethyl sulfoxide and benzalkonium chloride, enhance drug permeation by disrupting the structure of the lipophilic membrane barrier and, thus, facilitate permeation of both lipophilic and hydrophilic drug molecules through the membrane (Dragicevic and Maibach 2015). CDs on the other hand enhance the concentration of dissolved drug molecules in the aqueous exterior, thereby increasing the concentration gradient over the membrane barrier (Loftsson and Brewster 2011). CDs have a negligible effect on the bioavailability of hydrophilic drugs but can have significant effects on the bioavailability of lipophilic and poorly-soluble drugs (Loftsson et al. 2016). The ability of drug/CD complexes to aggregate and form nanoparticles can result in site specific drug delivery such as topical drug delivery to the hair follicles (Konrádsdóttir et al. 2009).

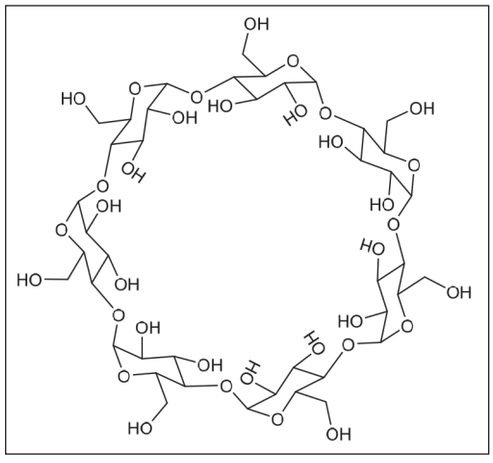

Fig. 1: The structure of the unsubstituted βCD showing the primary and secondary HO-groups located at the external rims of the molecule.

2. Self-assembly of cyclodextrins

According to their truncated conical shape, CDs are able to form host-guest inclusion complexes by including a hydrophobic moiety or a whole guest molecule into their hydrophobic central cavity (Fig. 2). Occasionally guest molecules form hydrogen bonds with the hydroxy groups at the rim of the CD cavity resulting in non-inclusion guest/CD complex formation (de Jesus et al. 2012; Loftsson et al. 2004). Those hydroxy groups can also form intermolecular CD-CD hydrogen bonds leading to self-assembly of dissolved CD molecules, that is formation of CD aggregates. CD complexes (i.e. inclusion and non-inclusion complexes) and self-assembled CD aggregates most often coexist in aqueous media but the currently applied analytical techniques do not readily detect these aggregates and, thus, their formation is most often neglected. The size of natural CD aggregates in aqueous solutions is about 140 to 300 nm. The size depends on the type of CD, the CD concentration, temperature and excipients present in the aqueous media. Furthermore, the methods applied to detect the aggregates can have effect on their size and size distribution. Applying dynamic light scattering (DLS) the size of the CD aggregates has been reported to be about 200 to 300 nm and the studies show that the aggregate size increases as a function of CD concentration (Coleman et al. 1992). CD solutions passed through 0.22 μm membrane filter show monodisperse aggregates while the unfiltrated CD solutions show two populations, a monomeric CD population (or non-aggregates which has mean hydrodynamic diameter of less than 1 nm) and a population of CD aggregates (Gonzalez-Gaitano et al. 2002; Wu et al. 2006a). Studies of formation of γCD aggregates and their hydrodynamic diameter, applying diffusion measurements, indicate that there is no clear relationship between their size and the aggregate formation tendency (Ribeiro et al. 2008). In aqueous solutions, an equilibrium exists between aggregates and unaggregated CD molecules but unaggregated CD molecules are dominating. In pure aqueous CD solutions the mass of aggregates is about 0.8% in 12 mM aqueous αCD solution (Gonzalez-Gaitano et al. 2002), 0.0011% in 10 mM βCD solution (Wu et al. 2006b) and 0.02% in 12 mM γCD solution (Szente et al. 1998). Furthermore, CD aggregates will dissociate when CD-CD H-bonds are disrupted, for example, through ionization or substitution of the OH-moieties or by addition of chaotropic agents to the aqueous complexation media. When pH of an aqueous βCD solution is greater than 12.5 or greater than 10 for that of a γCD solution, the hydrodynamic diameter of aggregates dramatically decreases due to the ionization of OH groups (Coleman et al. 1992). Substitution the βCD's OH-moieties with methyl- or hydroxypropyl groups results in significant decrease in the aggregate size (Coleman et al. 1992; Gonzalez-Gaitano et al. 2002). Presence of chaotropic agents, such as urea or potassium thiocyanate, in aqueous CD solutions interferes with formation of hydrogen bonds resulting in decreasing aggregate diameter of γCD (Szente et al. 1998) and HPβCD (Häusler and Müller-Goymann 1993). These observations indicate that CD aggregates are formed by intermolecular H-bonds. The shapes of CD aggregates have been studied by various microscopic techniques. Spherical aggregates have been observed in aqueous αCD and γCD solutions by transmission electron microscopy (TEM) (Polarz et al. 2001; Wu et al. 2006a). Morphological variations of βCD aggregate have been determined using TEM at cryogenic temperature (Cryo-TEM). When concentration of βCD increases, morphology of aggregates changes. Polyhedral aggregates forming a branched structure, larger globular particles and sheet-like aggregates have been observed in aqueous 3, 6 and 12 mM βCD solutions, respectively. The large bidimensional sheets observed in aqueous 12 mM βCD solutions become entangled to form long fibers and folded lamellaes upon sonication (Bonini et al. 2006a).

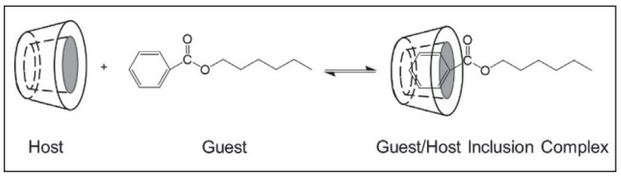

Fig. 2: Formation of guest (e.g., drug) – host (i.e. CD) inclusion complex.

The tendency of CDs to self-assemble to form aggregates is described by the critical aggregation concentration (cac) that is defined as the lowest CD concentration at which aggregates can be detected under some specific conditions (e.g., temperature) (Fülöp et al. 2015). The cac of the natural αCD, βCD and γCD in pure aqueous solutions and ambient temperature is about 25, 8 and 9 mg/ml, respectively (Saokham et al. 2016; Sá Couto et al. 2018c). Due to its low aqueous solubility (Table 1) βCD has a high tendency to form aggregates (i.e. βCD has the lowest cac value). Upon storage, aqueous γCD solutions become opalized and in some cases precipitation of γCD is observed (Szente et al. 1998). The cac of 2-hydroxypropyl-β-cyclodextrin (HPβCD), a randomly substituted derivative of βCD, in pure aqueous solutions is about 118 mg/ml (Sá Couto et al. 2018a) indicating the influence of random substitution of the βCD OH-moieties on the aggregation tendency. The presence of urea is reported to increase the cac value of αCD and γCD (Sá Couto et al. 2018b). Formation of guest/CD complexes can increase or decrease the cac values. Since transient CD aggregates are formed through hydrogen bonding of proton atoms located outside the CD cavity, self-assembly tendency decreases or increases upon guest/CD inclusion complex formation that can either hinder or facilitate intermolecular hydrogen bonding between CD molecules (Saokham et al. 2018a; Saokham and Loftsson 2015; Jansook et al. 2010b).

3. How to detect CD nano-aggregates?

Self-assembly of CD molecules can decrease their ability to form inclusion complexes with drugs, and since the natural CDs have a stronger tendency to aggregate than their derivatives this effect can

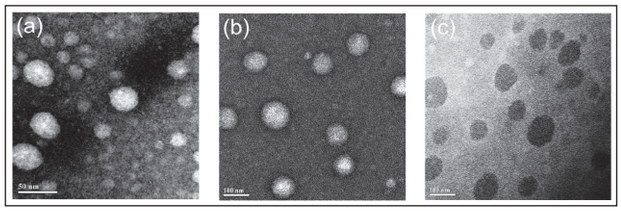

Fig. 3: TEM micrographs of nanoaggregates drug/CD complexes; hydrocortisone (a), amphotericin B (b), cyclosporin A (c).

be more dominating in their case (Wu et al. 2006a; Messner et al. 2010). Thus, it is important to know how the aqueous complexation media and external factors such as temperature affect the aggregation. Aggregation of βCD was investigated by cac measurements by Bonini et al. (2006b). The presence of αCD or γCD aggregates can be determined by simple visual observations, such as by the solution turbidity (Koichiro et al. 1983; Sá Couto et al. 2018b). Turbid CD solutions can become clear upon filtration only to be become turbid again upon storage (Szente et al. 1998). Formation of such particulate matter in aqueous CD solutions are frequently unacceptable in pharmaceutical products, for example in parenteral solutions. However, the formation of nano-size aggregates (diameter commonly between 20 and 300 nm) can enhance CD solubilization of water-insoluble drugs through formation of micellar-like structures or CD superstructures. The most common methods to detect nano-size aggregates of CDs and their complexes are dynamic light scattering (DLS) and transmission electron microscopy (TEM). Other methods include measurements of conductivity, osmolality, viscosity, surface tension, mass spectrometry, nuclear magnetic resonance, and the permeation through semi-permeable membranes. The principles, the advantages and the limitation of these methods have previously been reviewed (Messner et al. 2010; Jansook et al. 2018b).

DLS determines the hydrodynamic radius of the particles. DLS studies have shown that, in general, there are two size populations in aqueous CD solutions representing the monomeric CDs with diameter of less than 1 nm and small self-assembly of CD molecules ranging from 10-90 nm to a few hundred nm (Bonini et al. 2006b; Coleman et al. 1992; Gonzalez-Gaitano et al. 2003; He et al. 2008; Kashapov et al. 2017; Huang et al. 2019). In case of drug/CD complexes, three size populations are frequently observed in aqueous solutions, including large aggregates with diameter that can be from 100-500 nm to a few thousand nm (Muankaew et al. 2014; Do et al. 2017). TEM analysis has been used to support the DLS results. In general, TEM images show somewhat smaller aggregate sizes than DLS (Fig. 3). In aqueous solutions the particle size and the size distribution depend on the CD concentration, and factors such as media composition and temperature. Lucio et al. (2017) used DLS to measure the aggregate size in glibenclamide saturated CD solutions at various CD concentrations. At low CD concentrations (<5 mM) unimodal population was detected that corresponded to the monomeric CDs, while at higher CD concentrations bimodal distribution was observed corresponding to the monomeric CDs and small aggregates with a mean diameter of around 50 nm (Lucio et al. 2017). DLS data and TEM studies have shown that when thermal methods (i.e. autoclaving or sonication under heating) are applied during preparation of ternary drug/CD/polymer complexes an enhanced drug solubility is obtained through the formation of polymer stabilized drug/CD nanoaggregates (Jansook et al. 2018a).

Garnero et al. (2010) investigated aggregate formation in pure aqueous HPβCD solution and a solution containing the trimethoprim/HPβCD complex by conductivity measurements. The cac was determined by observing the specific conductivity change as a function of HPβCD concentration. The negative deviation from linearity demonstrated formation of HPβCD aggregates which occurred above the cac value. ^{1}H NMR spectroscopy can be used to determine the CD aggregate formation from the chemical shift data. The chemical shift is then plotted as a function of the inverse total CD concentration. Applying this method the cac value was determined from the intersection of the linear portions of the plot at the concentration above and below the inflection region (Duan et al. 2005; Garnero and Longhi 2007). Another suitable method for detection of CD aggregates is osmolality measurements with activity coefficient determinations. Deviation of the activity coefficient from ideality indicates CD aggregate formation (Jansook et al. 2010a). Sá Couto et al. (2018d) studied various techniques to detect the formation of CD aggregates. They found that both viscosity and surface tension determinations were unsuitable cac while the permeation study was the most reliable method for detection of the transient CD aggregates. The formation of aggregates increased with increasing CD concentration. The apparent cac values of αCD and βCD solutions were determined by this technique and shown that βCD has higher tendency to form aggregates than αCD (Sá Couto et al. 2018d). Jansook et al. investigated and demonstrated that the permeation flux profiles deviated from the linearity due to self-assemble of drug/CD complexes (Jansook and Loftsson 2009, Jansook et al. 2010a). Messner et al. (2011) estimated the fraction of aggregated complexes in saturated hydrocortisone/CD solutions by drug permeation through semipermeable membranes of different molecular weight cutoff (MWCO). Furthermore, they proposed some aggregation mechanisms and concluded that aggregation of CD molecules and the CD complexes is spontaneous and highly dynamic (Messner et al. 2011; Do et al. 2017).

4. Pharmacokinetics and toxicological considerations

CDs that are currently used in drug products are relatively large (molecular weight from about 1000 to just over 2000 Da) and hydrophilic ($LogP_{octanol/water}$ between -17 and -6) and, thus, do not readily permeate lipophilic biomembranes such as mucosa. The pharmacokinetics of CDs is similar to those of low molecular weight dextrans (Loftsson et al. 2016; Jansook et al. 2018b; Loftsson 2015). After oral administration CDs are digested by bacteria in the gastrointestinal tract or, in case of the natural γCD, by human α-amylase with only minor amounts being absorbed intact. The oral bioavailability of CDs that are currently used in pharmaceutical products is generally below 2% (Jansook et al. 2018b). Only RMβCD has higher oral bioavailability or as high as 12% (Loftsson and Brewster 2011). After intravenous administration the hydrophilic CDs (e.g., HPβCD and SBEβCD) are rapidly excreted unchanged with the urine with a half-life of less than 2 h. Also, due to their very hydrophilic nature these CDs have relatively small volume of distribution or less than 0.2 L/kg.

According to the European Medicines Agency (EMA), CDs are safe to use as excipients in pharmaceutical products (EMA 2017). However, like other carbohydrates CDs at high oral doses (more than 1 g/kg/day) may cause diarrhea. The parent CDs (i.e. the natural αCD, βCD and γCD) are listed as generally regarded as safe (GRAS) by the US Food & Drug Administration (US FDA), while SBEβCD and HPβCD are accepted as Inactive Pharmaceutical Ingredients. In marketed drug products the total daily dose of orally administered αCD, βCD, γCD and HPβCD are 6, 0.5, 10 and 8 g/kg/day, respectively (Loftsson and Brewster 2010). Although some studies have indicated that CDs can irritate mucosa, suppositories containing high doses of βCD and HPβCD do not irritate rectal mucosa in human and rabbit studies, respectively (Irie and Uekama 1997). Various studies in humans have shown that CDs are as safe as other natural oligosaccharides. Patch tests in human volunteers have shown that βCD does not induce irritation or allergic dermatitis. Moreover antigenicity, mutagenicity and topical irritation studies show that HPβCD is safe (Irie and Uekama 1997). In an ophthalmic preparation, αCD at concentration higher than 4% caused superficial epithelial toxicity in rabbit cornea. Although a topical administration of 5% randomly methylated βCD (RMβCD) to the eye has been shown to cause conjunctival and corneal irritation in rabbits, there is an eye drop product containing RMβCD on the market (Clorocil®, Oftalder, Portugal) (Loftsson and Stefánsson 2002). Solutions containing 10% SBEβCD (Irie and Uekama 1997) and 12.5% HPβCD (Stella and He 2008) have been shown to be non-toxic and non-irritating in

Table 2: Some examples of recently drug delivery formulation containing CD nano- (diameter commonly between 50 and 300 nm), microaggregates (diameter commonly between 1 and 10 μm) and investigated results

Drug	CD	Final formulation	Targeted drug delivery	Preparation method	Results
Curcumin	HPγCD	Aqueous solution	Topical	Shaking	Curcumin/HPγCD was detected deeper into the epidermal cell. (Konrádsdóttir et al. 2009)
	βCD/EDA	Aqueous solution	Ocular	Solvent evaporation	The formulation improved corneal permeation and 3 h accumulative flux. (Liu et al. 2020)
Dexamethasone	γCD, HPγCD	Suspension	Ocular	Heating	The formulation showed to deliver drug to vitreous humor and retina in rabbits. (Loftsson et al. 2007, Jansook et al. 2010a)
Irbesartan	γCD, HPγCD	Suspension	Ocular	Heating	Self-aggregation was found, and increased permeation flux observed in the unionized inclusion complexes (Muankaew et al. 2014, Jansook et al. 2015)
Dorzolamide	γCD, HPγCD	Suspension Aqueous gel	Ocular	Heating Chemically crosslinking	The formulation enhanced drug delivery to the posterior segment of the eye.(Jansook et al. 2010c) The formulation gave mucoadhesive effect and improved drug delivery into the eye. (Moya-Ortega et al. 2012)
Voriconazole	NR	aqueous solution	Ocular	lyophilization	The formulation enhanced corneal penetration better than chitosan based formulation and liposome. (Gelfuso et al. 2019)
Vancomycin	βCD/OLA	Nanovesicles	NR	Inclusion complex suspension	The formulation could prolong the time of drug exposure to bacteria. (Salih et al. 2020)
Doxorubicin	βCD/PEG	Aqueous solution	NR	Solvent evaporation	Provide sustained release effect and increase antitumor activity. (Li et al. 2020)
Paclitaxel	HPβCD	Nanosphere suspension	Intravenous	Chemically crosslinking	Formulation enhanced drug transportation. (Tan et al. 2016)
Triamcinolone acetonide	HPβCD/PLGA	Suspension	Ocular	Quasi-emulsion solvent evaporation	Improve corneal penetration by increasing dispersion in the tear film. (Li et al. 2019)
Warfarin	βCD/chitosan	Aqueous solution	Transdermal	Chemically crosslinking	Increase stratum corneum fluidity and enhanced drug permeability. (Khalil et al. 2012)
Epalrestat	βCD, MEβCD	Suspension	Transdermal	Cogrinding	The formulation containing βCD derivatives showed strong enhancement of drug permeation through hairless mouse. (Furuishi et al. 2017)
Diclofenac	HPβCD/compritol ATO 888	Solid lipid nanoparticles	Colon	Hot homogenization	CD improved entrapment of drug and dissolution rate resulting in enhanced permeation performance. (Spada et al. 2012)

EDA = ethylene diamine; PEG = poly ethylene glycol; OLA = oleylamine
NR = not reported

rabbit eyes. Intravenous administration of αCD, βCD and RMβCD has resulted in some renal toxicity even at relatively low dose indicating that they are not suitable for parenteral products (Stella and He 2008). High doses of parenterally administered γCD caused slightly impairment of renal function in rats but no degenerative changes in kidneys were observed and the vacuolization was fully reversible after treatment (Donaubauer et al. 1998). Unlike the natural βCD, modified βCDs such as SBEβCD and HPβCD are considered safe to use in parenteral formulations at doses as high as 16 and 14 g/day, respectively. Intravenous administration of 250 mg/kg/day of HPβCD and SBEβCD for 21 days and 6 months, respectively, is safe in humans older than 2 years (Lee et al. 2015, Tolbert et al. 2015).

In general, CD aggregates are transient structures that are in dynamic equilibrium with monomeric CDs and CD complexes and, thus, CD aggregates do not affect the pharmacokinetics and toxicology of CDs after parenteral administration. This is due to the fact that the aggregates rapidly dissociate upon media dilution such as upon mixing with blood plasma. However, formation of CD aggregates can affect drug distribution upon topical administration where little or no dilution occurs such as upon dermal application or topical administration to the eye.

5. Drug delivery

Therapeutic drug efficacy depends mainly on the drug concentration at the target site. In our body, mucus layers (i.e. the aqueous exterior layer of mucosa) are serving as the first protective physical barrier against microorganisms and toxic substances and is as well a diffusion barrier for drug absorption into the body. Mucin is its main component and gives mucus the viscous gel-like property (Leal et al. 2017). CDs can act as mucus permeation enhancers for topical and oral drug delivery (Srichana et al. 2001; Francois et al. 2003). The theoretical background on how CDs and their inclusion complexes enhance drug permeation across biomembranes has been described by several authors (Brewster et al. 2007; Dahan et al. 2010; Loftsson 2012). Formation of CD aggregates has been associated with the permeation enhancement effect of CD as those small CD clusters can form inclusion complexes with free drug molecules in aqueous solution. This boosts the amount of solubilized drugs that are ready to permeate the mucus layer (Saokham et al. 2018b). Interestingly, the drug/CD complex nanoparticles and microparticles have been shown to be more effective drug permeation enhancers than the individual inclusion complexes (Loftsson 2014). In addition, the particles can result in controlled drug release (Salústio et al. 2011) and increased drug residence time at the site of delivery (Table 2). Accordingly, CD aggregates tend to behave more like nanosystems rather than true solutions. However, it should be noted that not all CD aggregates result in enhanced drug permeation. For example, large CD aggregates and hydrophobic CDs may prevent drug release from drug formulations (Nguyen et al. 2016).

Drug delivery systems containing CD aggregates can be classified into two main groups: 1) nanoparticles and microparticles consisting of aggregated CDs and/or inclusion complexes (Messner et al. 2010; Gudmundsdottir et al. 2014) and 2) CD-based formulations containing those aggregates as well as other components such as polymers, fatty acids and metals (Alvarez-Lorenzo et al. 2017; Menezes et al. 2019). In the latter group, formulations are most frequently designed for some specific targeted drug delivery. Regarding to CD types, natural CDs and their derivatives are generally selected to produced formulations in the first group. For instance, aqueous curcumin/HPγCD formulation targets the hair follicles due to spontaneous formation of curcumin/HPγCD nanoparticles (Konrádsdóttir et al. 2009). A voriconazole/CD formulation has displayed enhanced corneal penetration, in comparison to voriconazole/chitosan and liposomal formulations (Gelfuso et al. 2019). A microparticle eye drop formulation containing dexamethasone/γCD complex nano-aggregates has been shown sustained drug release and enhanced drug delivery

into the eye by enhancing dexamethasone delivery through the aqueous tear film that acts as aqueous diffusion barrier to topical drug delivery to the eye (Loftsson and Stefansson 2007; Jansook et al. 2010a). Similar results were observed in the case of dorzolamide/CD (Jansook et al. 2010c) and irbesartan/CD eye drop formulations (Muankaew et al. 2014; Jansook et al. 2015). Similar technology was used to enhance cyclosporin A delivery into eye tissues after topical administration (Jóhannsdóttir et al. 2015). These observations can be explained by the enhanced drug bioavailability obtained by formation of drug/CD complex aggregates. CDs are also used as building blocks for nanoparticle and microparticle drug carriers of some novel morphology. For examples, CD-based nanogels for ocular drug delivery was investigated by Moya-Ortega and co-workers and shown to enhance drug absorption (Moya-Ortega et al. 2013). The incorporation of inclusion complexes into a polymeric hydrogel network provides for high drug loading formulation. βCD/PEG aggregates synthesized by Li et al. (2020) have shown to improve antitumor drug delivery and activity. The micelle-like structures and liposomes composed of amphiphilic CD have exhibited sustained drug release and enhanced drug delivery to the targeted tumor (Zerkoune et al. 2014). Self-assembled nanoparticles of CD-grafted polymers as gene delivery system has also been reported by several authors (Fan et al. 2011; Lu et al. 2020). Some recent drug delivery formulations that are based on aggregated CDs and their permeation effect are summarized in Table 2. For more information readers are referred to couple recent publications (Conte et al. 2016; Ben Mihoub et al. 2018).

6. Conclusions

CDs and drug/CD complexes self-assembly to form transient nanoparticles and small microparticles. Formation of such particulate matter in aqueous CD solutions can lead to erroneous analytical results and product rejections during drug manufacturing. However, they have also given rise to formation of novel drug delivery systems with exceptional properties.

Conflicts of Interest: The authors declare no conflict of interest.

References

Alvarez-Lorenzo C, García–González CA, Concheiro A (2017) Cyclodextrins as versatile building blocks for regenerative medicine. J Control Release 268: 269–281.

Ben Mihoub A, Larue L, Moussaron A, Youssef Z, Colombeau L, Baros F, Frochot C, Vanderesse R, Acherar S (2018) Use of cyclodextrins in anticancer photodynamic therapy treatment. Molecules 23: 1936.

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

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

Brewster ME, Noppe M, Peeters J, Loftsson T (2007) Effect of the unstirred water layer on permeability enhancement by hydrophilic cyclodextrins. Int J Pharm 342: 250–253.

Coleman A, Nicolis I, Keller N, Dalbiez J (1992a) Aggregation of cyclodextrins: An explanation of the abnormal solubility of β–cyclodextrin. J Incl Phenom Macro 13: 139–143.

Conte C, Scala A, Siracusano G, Sortino G, Pennisi R, Piperno A, Miro A, Ungaro F, Sciortino M T, Quaglia F, Mazzaglia A (2016) Nanoassemblies based on non–ionic amphiphilic cyclodextrin hosting Zn(II)–phthalocyanine and docetaxel: Design, physicochemical properties and intracellular effects. Colloids and Surfaces B: Biointerfaces 146: 590–597.

Dahan A, Miller JM, Hoffman A, Amidon GE, Amidon GL (2010) The solubility–permeability interplay in using cyclodextrins as pharmaceutical solubilizers: mechanistic modeling and application to progesterone. J Pharm Sci 99: 2739–2749.

De Jesus MB, Fraceto LF, Martini MF, Pickholz M, Ferreira CV, De Paula E (2012) Non–inclusion complexes between riboflavin and cyclodextrins. J Pharm Pharmacol 64: 832–842.

Do TT, Van Hooghten R, Van Den Mooter G (2017) A study of the aggregation of cyclodextrins: Determination of the critical aggregation concentration, size of aggregates and thermodynamics using isodesmic and K2-K models. Int J Pharm 521: 318–326.

Dodziuk H (2006) Molecules with holes – cyclodextrins. In: Dodziuk H (Ed.) Cyclodextrins and Their Complexes: Chemistry, Analytical Methods, Applications, Wiley, Weinheim.

Donaubauer HH, Fuchs H, Langer KH, Bär A (1998) Subchronic intravenous toxicity studies with γ-cyclodextrin in rats. Reg Toxicol Pharmacol 27: 189–198.

Dragicevic N, Maibach HI (2015) Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement, Springer Berlin Heidelberg.

Duan M S, Zhao N, Össurardóttir Í B, Thorsteinsson T, Loftsson T (2005) Cyclodextrin solubilization of the antibacterial agents triclosan and triclocarban: Formation of aggregates and higher-order complexes. Int J Pharm 297: 213–222.

E M A (2017) Cyclodextrins used as excipients.

Fan M-M, Zhang X, Qin J, Li B--, Sun X, Zhang S (2011) Self–assembly Pluronic and β-cyclodextrin to hollow nanospheres for enhanced gene delivery. Macromol Rapid Comm 32: 1533–1538.

Francois M, Snoeckx E, Putteman P, Wouters F, De Proost E, Delaet U, Peeters J, Brewster MP (2003) A mucoadhesive, cyclodextrin–based vaginal cream formulation of itraconazole. AAPS pharmSci 5: E5–E5.

Fülöp Z, Balogh A, Saokham P, Jansook P, Loftsson T (2015) Formation and stability assessment of self-assembled nanoparticles from large Mw chitosan and sulfobutylether-β-cyclodextrin. J Drug Deliv Sci Technol B 30: 478-485.

Furuishi T, Takahashi S, Ogawa N, Gunji M, Nagase H, Suzuki T, Endo T, Ueda H, Yonemochi E, Tomono K (2017) Enhanced dissolution and skin permeation profiles of epalrestat with β–cyclodextrin derivatives using a cogrinding method. Eur J Pharm Sci 106: 79–86.

Garnero C, Longhi M (2007) Study of ascorbic acid interaction with hydroxypropyl-β-cyclodextrin and triethanolamine, separately and in combination. J Pharm Biomed Anal 45: 536–545.

Garnero C, Zoppi A, Genovese D, Longhi M (2010) Studies on trimethoprim:hydroxypropyl-β-cyclodextrin: aggregate and complex formation. Carbohydr Res 345: 2550–2556.

Gelfuso GM, Ferreira-Nunes R, Dalmolin LF, Dos S Ré AC, Dos Santos GA, De Sá FAP, Cunha-Filho M, Alonso A, Mendanha Neto S A, L. V. Anjos J, Aires C P, F. V. Lopez R, Gratieri T (2019) Iontophoresis enhances voriconazole antifungal potency and corneal penetration. Int J Pharm 576: 118991.

Gonzalez–Gaitano G, Rodriguez P, Isasi J R, Fuentes M, Tardajos G, Sanchez M (2002) The aggregation of cyclodextrins as studied by photon correlation spectroscopy. J Incl Phenom Macro 44: 101–105.

Gudmundsdottir BS, Petursdottir D, Asgrimsdottir GM, Gottfredsdottir MS, Hardarson SH, Johannesson G, Kurkov SV, Jansook P, Loftsson T, Stefansson E (2014) γ-Cyclodextrin nanoparticle eye drops with dorzolamide: effect on intraocular pressure in man. J Ocul Pharmacol Ther 30: 35–41.

Häusler O, Müller-Goymann CC (1993) Properties and structure of aqueous solutions of hydroxypropyl-beta-cyclodextrin. Starch–Starke 45: 183–187.

He Y, Fu P, Shen X, Gao H (2008) Cyclodextrin-based aggregates and characterization by microscopy. Micron 39: 495–516.

Huang T, Zhao Q, Su Y, Ouyang D (2019) Investigation of molecular aggregation mechanism of glipizide/cyclodextrin complexation by combined experimental and molecular modeling approaches. Asian J Pharm Sci 14: 609–620.

Irie T, Uekama K (1997) Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. J Pharm Sci 86: 147–162.

Jansook P, Kulsirachote P, Loftsson T (2018a) Cyclodextrin solubilization of celecoxib: solid and solution state characterization. J Incl Phenom Macro 90: 75–88.

Jansook P, Loftsson T (2009) CDs as solubilizers: Effects of excipients and competing drugs. Int J Pharm 379: 32–40.

Jansook P, Kurkov SV, Loftsson T (2010a) Cyclodextrins as solubilizers: Formation of complex aggregates. J Pharm Sci 99: 719–729.

Jansook P, Moya-Ortega MD, Loftsson T (2010b) Effect of self–aggregation of γ–cyclodextrin on drug solubilization. J Incl Phenom Macro 68: 229–236.

Jansook P, Stefánsson E, Thorsteinsdóttir M, Sigurdsson BB, Kristjánsdóttir SS, Bas J, Sigurdsson HH, Loftsson T (2010c) Cyclodextrin solubilization of carbonic anhydrase inhibitor drugs: Formulation of dorzolamide eye drop microparticle suspension. Eur J Pharm Biopharm 76: 208–214.

Jansook P, Muankaew C, Stefánsson E, Loftsson T (2015) Development of eye drops containing antihypertensive drugs: formulation of aqueous irbesartan/γCD eye drops. Pharm Devel Technol 20: 626–632.

Jansook P, Ogawa N, Loftsson T (2018b) Cyclodextrins: structure, physicochemical properties and pharmaceutical applications. Int J Pharm 535: 272–284.

Jóhannsdóttir S, Jansook P, Stefánsson E, Loftsson T (2015) Development of a cyclodextrin-based aqueous cyclosporin A eye drop formulations. Int J Pharm 493: 86–95.

Kashapov RR, Mamedov VA, Zhukova NA, Kadirov MK, Nizameev IR, Zakharova LY, Sinyashin OG (2017) Controlling the binding of hydrophobic drugs with supramolecular assemblies of β-cyclodextrin. Colloids Surfaces A 527: 55–62.

Khalil SKH, El-Feky GS, El-Banna ST, Khalil WA (2012) Preparation and evaluation of warfarin–β–cyclodextrin loaded chitosan nanoparticles for transdermal delivery. Carbohydr Polym 90: 1244–1253.

Koichiro M, Masahiro S, Masayuki N (1983) Viscosity B-coefficients, apparent molar volumes, and activity coefficients for α- and γ-cyclodextrins in aqueous solutions. Bull Chem Soc Japan 56: 3556–3560.

Konrádsdóttir F, Ogmundsdóttir H, Sigurdsson V, Loftsson T (2009) Drug targeting to the hair follicles: a cyclodextrin–based drug delivery. AAPS PharmSciTech 10: 266–269.

Leal J, Smyth HDC, Ghosh D (2017) Physicochemical properties of mucus and their impact on transmucosal drug delivery. Int J Pharm 532: 555–572.

Lee D, Kalu U, Halford J J, Biton V, Cloyd J, Klein P, Bekersky I, Peng G, Dheerendra S, Tolbert D (2015) Intravenous carbamazepine as short–term replacement therapy for oral carbamazepine in adults with epilepsy: Pooled tolerability results from two open-label trials. Epilepsia 56: 906–914.

Li F, Wen Y, Zhang Y, Zheng K, Ban J, Xie Q, Wen Y, Liu Q, Chen F, Mo Z, Liu L, Chen Y, Lu Z (2019) Characterisation of 2-HP-β-cyclodextrin-PLGA nanoparticle complexes for potential use as ocular drug delivery vehicles. Artif Cells, Nanomed Biotechnol 47: 4097–4108.

Li J, Xin M, Huo Y, Cai A, Yan M, Wang C, Wei G (2020) Synthesis of β-cyclodextrin-PEG-G molecules to delay tumor growth and application of β-cyclodextrin-PEG-G aggregates as drug carrier. Carbohydr Polym 229: 115478.

Liu C-H, Lee G-W, Wu W-C, Wang C-C (2020) Encapsulating curcumin in ethylene diamine–β–cyclodextrin nanoparticle improves topical cornea delivery. Colloids and Surfaces B: Biointerfaces 186: 110726.
Loftsson T (2012) Drug permeation through biomembranes: cyclodextrins and the unstirred water layer. Pharmazie 67: 363–370.
Loftsson T (2014) Self-assembled cyclodextrin nanoparticles and drug delivery. J Incl Phen Macro 80: 1–7.
Loftsson T (2015) Excipient pharmacokinetics and profiling. Int J Pharm 480: 48–54.
Loftsson T, Brewster ME (2010) Pharmaceutical applications of cyclodextrins: basic science and product development. J Pharm Pharmacol 62: 1607–1621.
Loftsson T, Brewster ME (2011) Pharmaceutical applications of cyclodextrins: effects on drug permeation through biological membranes. J Pharm Pharmacol 63: 1119–1135.
Loftsson T, Brewster ME, Másson M (2004) Role of cyclodextrins in improving oral drug delivery. Am J Drud Deliv 2: 261–275.
Loftsson T, Hreinsdóttir D, Stefánsson E (2007) Cyclodextrin microparticles for drug delivery to the posterior segment of the eye: aqueous dexamethasone eye drops. J Pharm Pharmacol 59: 629–635.
Loftsson T, Moya-Ortega MD, Alvarez-Lorenzo C, Concheiro A (2016) Pharmacokinetics of cyclodextrins and drugs after oral and parenteral administration of drug/cyclodextrin complexes. J Pharm Pharmacol 68: 544–555.
Loftsson T, Saokham P, Couto AR- (2019) Self–association of cyclodextrins and cyclodextrin complexes in aqueous solutions. Int J Pharm 560: 228–234.
Loftsson T, Stefansson E (2007) Cyclodextrins in ocular drug delivery: theoretical basis with dexamethasone as a sample drug. J Drug Delivery Sci Technol. 17: 3–9.
Loftsson T, Stefánsson E (2002) Cyclodextrins in eye drop formulations: enhanced topical delivery of corticosteroids to the eye. Acta Ophthal Scand 80: 144–150.
Lu S, Bao X, Hai W, Shi S, Chen Y, Yu Q, Zhang M, Xu Y, Peng J (2020) Multi-functional self-assembled nanoparticles for pVEGF-shRNA loading and anti–tumor targeted therapy. Int J Pharm 575: 118898.
Lucio D, Irache JM, Font M, Martínez-Ohárriz MC (2017) Nanoaggregation of inclusion complexes of glibenclamide with cyclodextrins. Int J Pharm 519: 263–271.
Menezes PDP, Andrade TDA, Frank LA, De Souza EPBSS, Trindade GDGG, Trindade I a S, Serafini MR, Guterres SS, Araújo A a DS (2019) Advances of nanosystems containing cyclodextrins and their applications in pharmaceuticals. Int J Pharm 559: 312–328.
Messner M, Kurkov SV, Brewster ME, Jansook P, Loftsson T (2011) Self-assembly of cyclodextrin complexes: Aggregation of hydrocortisone/cyclodextrin complexes. Int J Pharm 407: 174–183.
Messner M, Kurkov S V, Jansook P, Loftsson T (2010) Self-assembled cyclodextrin aggregates and nanoparticles. Int J Pharm 387: 199–208.
Moya-Ortega MD, Alvarez-Lorenzo C, Sigurdsson HH, Concheiro A, Loftsson T (2012) Cross-linked hydroxypropyl-β-cyclodextrin and γ-cyclodextrin nanogels for drug delivery: Physicochemical and loading/release properties. Carbohydr Polym 87: 2344–2351.
Moya-Ortega MD, Alves TFG, Alvarez-Lorenzo C, Concheiro A, Stefánsson E, Thorsteinsdóttir M, Loftsson T (2013) Dexamethasone eye drops containing γ-cyclodextrin-based nanogels. Int J Pharm 441: 507–515.
Muankaew C, Jansook P, Stefánsson E, Loftsson T (2014) Effect of γ-cyclodextrin on solubilization and complexation of irbesartan: influence of pH and excipients. Int J Pharm 474: 80–90.
Nguyen KTH, Mathias EV, Porter E, Ba Y (2016) Diffusions of β-cyclodextrins in mucus studied by 19F diffusion NMR. J Incl Phen Macro 86: 273–282.
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.
Ribeiro ACF, Santos CI a V, Valente AJM, Ascenso OS, Lobo VMM, Burrows HD, Cabral AMTDPV, Veiga F, Teijeiro C, Esteso MA (2008) Some transport properties of γ-cyclodextrin aqueous solutions at (298.15 and 310.15) K. J Chem Engin Data 53: 755–759.
Salih M, Omolo CA, Agrawal N, Walvekar P, Waddad AY, Mocktar C, Ramdhin C, Govender T (2020) Supramolecular amphiphiles of beta-cyclodextrin and oleylamine for enhancement of vancomycin delivery. Int J Pharm 574: 118881.
Salústio PJ, Pontes P, Conduto C, Sanches I, Carvalho C, Arrais J, Marques HMC (2011) Advanced technologies for oral controlled release: cyclodextrins for oral controlled release. AAPS PharmSciTech 12: 1276–1292.
Saokham P, Couto AS, Ryzhakov A, Loftsson T (2016) The self-assemble of natural cyclodextrins in aqueous solutions: application of miniature permeation studies for critical aggregation concentration (cac) determinations. Int J Pharm 505: 187–193.
Saokham P, Do TT, Van Den Mooter G, Loftsson T (2018a) Inclusion complexes of p–hydroxybenzoic acid esters and γ-cyclodextrin. J Incl Phen Macro 90: 111–122.
Saokham P, Loftsson T (2015) A new approach for quantitative determination of γ–cyclodextrin in aqueous solutions: application in aggregate determinations and solubility in hydrocortisone/γ-cyclodextrin inclusion complex. J Pharm Sci 104: 3925–3933.
Saokham P, Muankaew C, Jansook P, Loftsson T (2018b) Solubility of cyclodextrins and drug/cyclodextrin complexes. Molecules 23: 1161.
Sá Couto AR, Ryzhakov A, Loftsson T (2018a) 2-Hydroxypropyl-β-cyclodextrin aggregates: identification and development of analytical techniques. Materials 11: 1971.
Sá Couto AR, Ryzhakov A, Loftsson T (2018b) Disruption of α- and γ-cyclodextrin aggregates promoted by chaotropic agent (urea). J Drug Del Sci Technol 48: 209–214.
Sá Couto AR, Ryzhakov A, Loftsson T (2018c) Self-assemble of α-cyclodextrin and β-cyclodextrin: identification and development of analytical techniques. J Pharm Sci 107: 2208–2215.
Sá Couto AR, Ryzhakov A, Loftsson T (2018d) Self-assembly of b1-cyclodextrin and b2-cyclodextrin: identification and development of analytical techniques. J Pharm Sci 107: 2208–2215.
Spada G, Gavini E, Cossu M, Rassu G, Giunchedi P (2012) Solid lipid nanoparticles with and without hydroxypropyl-β-cyclodextrin: a comparative study of nanoparticles designed for colonic drug delivery. Nanotechnology 23: 095101.
Srichana T, Suedee R, Reanmongkol W (2001) Cyclodextrin as a potential drug carrier in salbutamol dry powder aerosols: the in–vitro deposition and toxicity studies of the complexes. Respirat Med 95: 513–519.
Stella V J, He Q (2008) Cyclodextrins. Toxicol Pathol 36: 30–42.
Szejtli J (1988) Cyclodextrin technology, Springer Netherlands.
Szente L, Szejtli J, Kis GL (1998) Spontaneous opalescence of aqueous γ-cyclodextrin solutions: complex formation or self–aggregation? J Pharm Sci 87: 778–781.
Tan J, Meng N, Fan Y, Su Y, Zhang M, Xiao Y, Zhou N (2016) Hydroxypropyl-β-cyclodextrin-graphene oxide conjugates: carriers for anti–cancer drugs. Mater Sci Engin C 61: 681–687.
Tolbert D, Cloyd J, Biton V, Bekersky I, Walzer M, Wesche D, Drummond R, Lee D (2015) Bioequivalence of oral and intravenous carbamazepine formulations in adult patients with epilepsy. Epilepsia 56: 915–923.
Wu A, Shen X, He Y (2006a) Investigation on γ-cyclodextrin nanotube induced by N,N'-diphenylbenzidine molecule. J Colloid Interface Sci 297: 525–533.
Wu A, Shen X, He Y (2006b) Micrometer–sized rodlike structure formed by the secondary assembly of cyclodextrin nanotube. J Colloid Interface Sci 302: 87–94.
Zagami R, Romeo A, Mazzaglia A (2019) Bio–soft cyclodextrin nanomaterials. Rivista Del Nuovo Cimento 42: 407–441.
Zerkoune L, Angelova A, Lesieur S (2014) Nano-assemblies of modified cyclodextrins and their complexes with guest molecules: incorporation in nanostructured membranes and amphiphile nanoarchitectonics design. Nanomaterials 4: 741–765.