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Recent Aspect of Cyclodextrin-Based Drug Delivery System

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Key words: absorption enhancement, conjugate, controlled drug release, cyclodextrin, drug carrier, inclusion complex, pharmaceutical formulation, targeting

Abstract

The pharmaceutically useful cyclodextrins (CyDs) are classified into hydrophilic, hydrophobic, and ionic derivatives. These CyDs can serve as multi-functional drug carriers, through the formation of inclusion complex or the form of CyD/drug conjugate. In addition, the combined use of different CyDs and/or pharmaceutical excipients is capable of alleviating the undesirable properties of drug molecules, improving efficacy and reducing side effects. This contribution outlines the potential use of CyDs in the design and evaluation of CyD-based drug formulation, focusing on their ability to enhance the drug absorption across biological barriers, the ability to control the rate and time profiles of drug release, and the ability to deliver a drug to targeted site.

Introduction

The desirable attributes of drug carriers in drug delivery system are the multi-functional properties such as controlled-release, targeting, and absorption enhancing abilities [1, 2]. From the safety viewpoint, bioadaptability is an important necessity, and high quality, cost-performance, etc. are required for drug carriers. CyDs have such characteristics; e.g. they are fairly bioadaptable and hardly absorbable from gastrointestinal (GI) tracts, they interact with specific components of biomembrane such as cholesterol and lipids, their macrocyclic ring survives in stomach and small intestine, but they are biodegradable in colon and large intestine, and more functional CyD derivatives are available to modify the physicochemical and inclusion properties of the host molecules [3–5]. Table 1 contains the pharmaceutically useful β-CyD derivatives, classified into hydrophilic, hydrophobic, and ionic derivatives [2]. Some hydrophilic CyDs have been practically applied in the pharmaceutical preparations [6, 7]. The hydrophobic CyDs may serve as sustained release carriers for the water-soluble drugs including peptide and protein drugs [2, 8]. The delayed release formulation can be obtained by the use of enteric type CyDs such as *O*-carboxymethyl-*O*-ethyl-β-CyD (CME-β-CyD) [9]. A combined use of different CyDs and/or pharmaceutical additives will provide more balanced oral bioavailability with prolonged therapeutic effects [2]. The desirable attribute for the drug carrier is its ability to deliver a drug to targeted site. The CyD/drug conjugate can be a versatile means of constructing a new class of colon-targeting prodrug [10]. On the basis of the above-mentioned knowledge, the advantages and limitations of CyDs in the design of advanced dosage forms will be discussed.

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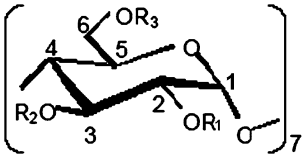
Enhancement of drug absorption

The possible enhancing mechanisms of CyDs on the bioavailability of drugs in various administration routes are summarized as follows: (1) hydrophilic CyDs increase the solubility, dissolution rate, and wettability of poorly water-soluble drugs; (2) CyDs prevent the degradation or disposition of chemically unstable drugs in gastrointestinal tracts as well as during storage; (3) CyDs perturb the membrane fluidity to lower the barrier function, which consequently enhances the absorption of drugs including peptide and protein drugs through the nasal and rectal mucosa; and (4) competitive inclusion complexation with third components (bile acid, cholesterol, lipids, etc.) to release the included drug. These four mechanisms are well recognized, and the factors affecting the drug absorption by hydrophilic CyDs have been extensively studied [2–5].

Recently, we have revealed the new enhancing mechanism of 2,6-di-*O*-methyl-β-CyD (DM-β-CyD) with respect to multidrug efflux pump, P-glycoprotein (P-gp) and multidrug resistant-associated protein 2 (MRP2) for oral bioavailability of hydrophobic drugs (e.g. tacrolimus, a typical P-gp substrate) in Caco-2 cell and vinblastine-resistant Caco-2 (Caco-2R) cell monolayers (Figure 1); i.e. DM-β-CyD enhances the oral

Table 1. Pharmaceutically useful β-CyDs



Derivative	Characteristic	Possible use (dosage form)
Hydrophilic derivatives		
Methylated β-CyD		
Me-β-CyD	Soluble in cold water and in organic solvents,	Oral, dermal, mucosal[a]
DM-β-CyD	Surface active, Hemolytic	
TM-β-CyD		
DMA-β-CyD	Soluble in water, Low hemolytic	Parenteral, oral, mucosal
Hydroxyalkylated β-CyD		
2-HE-β-CyD		Parenteral, oral, mucosal
2-HP-β-CyD	Amorphous mixture with different d. s. (Encapsin^{RT})	Parenteral, oral mucosal
3-HP-β-CyD	Highly water-soluble (> 50%), Low toxicity	Parenteral, oral, mucosal
2,3-DHP-β-CyD		Parenteral, oral, mucosal
Branched β-CyD		
G_1-β-CyD	Highly water-soluble (> 50%)	Parenteral, oral, mucosal
G_2-β-CyD	Low toxicity	Parenteral, oral, mucosal
GUG-β-CyD		Parenteral,, oral, mucosal
Hydrophobic derivatives		
Alkylated β-CyD		
DE-β-CyD	Water-insoluble, soluble in organic solvents, surface-active	Oral, subcutaneous (slow-release)
TE-β-CyD		
Acylated β-CyD		
TA-β-CyD	Water-insoluble, soluble in organic solvents	Oral, parenteral (slow-release)
TB-β-CyD	Mucoadhesive	(slow-release)
TV-β-CyD	Film formation.	(slow-release)
TO-β-CyD		
Ionizable derivatives		
Anionic β-CyD		
CME-β-CD	$pK_a = 3$–4, Soluble at pH > 4,	Oral, dermal, mucosal (delayed-release, enteric[b])
β-CyD sulfate	$pK_a > 1$, Water-soluble	Oral, mucosal
SBE4-β-CyD	Water-soluble	Parenteral, oral
SBE7-β-CyD	Water-soluble, (Captisol^{RT})	Parenteral, oral
A1-β-CyD sulfate	Water-insoluble	Parenteral (slow-release)

Abbreviations: Me: randomly-methylated; DM: 2,6-di-*O*-methyl; TM: 2,3,6-tri-*O*-methyl; DMA: acetylated DM-β-CyD; 2-HE: 2-hydroxyethyl; 2-HP: 2-hydroxypropyl; 3-HP: 3-hydroxypropyl; 2,3-DHP: 2,3-dihydroxypropyl; G_1: glycosyl; G_2: maltosyl; GUG: Glucuronyl-glucosyl; DE: 2,6-di-*O*-ethyl; TB: 2,3,6-tri-*O*-ethyl; CME: *O*-carboxymethyl-*O*-ethyl; TA: 2,3,6-tri-*O*-acyl (C_2–C_{18}); TB:2,3,6-tri-*O*-butanoyl; TV: 2,3,6-tri-*O*-valeryl; TO: 2,3,6-tri-*O*-octyl; SBE4: d.s.4 of sulfobutyl ether group; SBE7 d.s.7 of sulfobutyl ether group.
[a]Mucosal: nasal, sublingual, ophthalmic, pulmonary, rectal, vaginal, etc.
[b]Enteric: soluble in intestinal fluid (pH 6–7).

bioavailability of hydrophobic drugs not only by its solubilizing effect but also its inhibitory effect on the efflux pump activity of P-gp and/or MRP2 [11]. Besides, DM-β-CyD suppresses the function of L-type amino acid transporter 1 and Na^+/glucose cotransporter 1 through the release of these transporters from the apical membranes of Caco-2R cell monolayers as well. Interestingly, DM-α-CyD augments H^+-coupled peptide transporter PepT1-mediated uptake of glycylsarcosine in Caco-2 and Caco-2R cell monolayers. Moreover, the hydrophilic CyDs might affect drug metabolism by cytochrome P450 (CYP) 3A, the major phase I drug metabolizing enzymes, which exist in both intestinal epithelial cells and hepatocytes. Thus, CyDs can enhance the oral bioavailability of drugs in different ways, and the enhancing mechanism of CyDs on oral bioavailability of drugs may be more complicated than we have so far believed.

Control of drug release

The plasma drug levels time profiles after oral administration can be classified into the rate-controlled release and the time-controlled release. The rate-controlled release is further classified into three types; i.e. immediate-release, prolonged-release and modified-release (Table 2). The hydrophobic CyDs such as ethylated and acylated CyDs with low aqueous solubility are known to

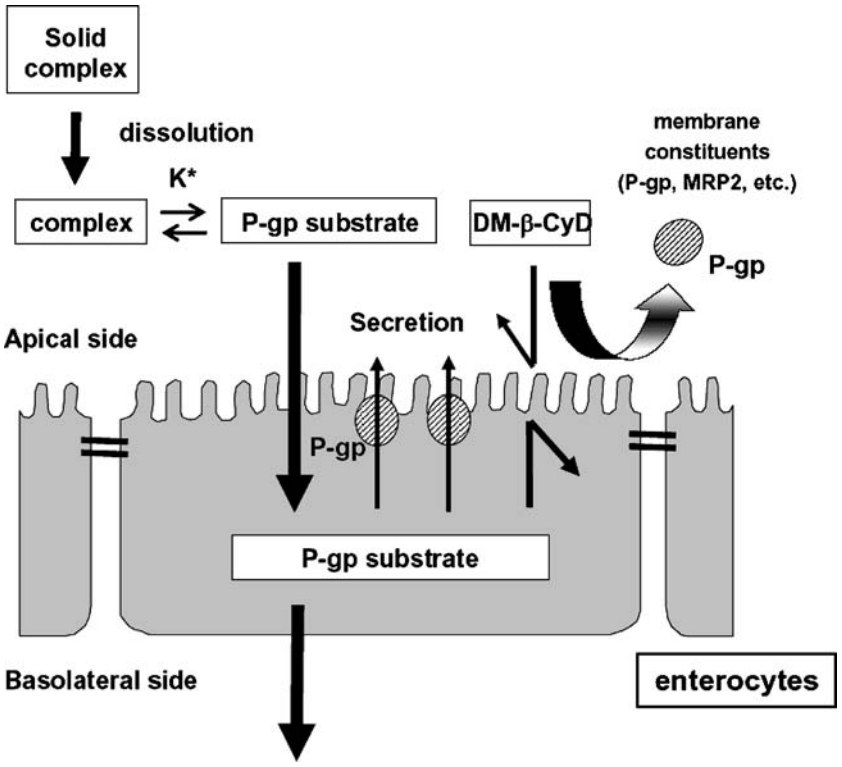


Figure 1. Possible enhancing mechanism of DM-β-CyD on oral bioavailability of hydrophobic drug, tacrolimus (P-gp substrate) [11]. *Stability constant of inclusion complex.

Table 2. Modification of drug release site and/or time profile by CyD

Release pattern	Aim	Use of CyD
Immediate release	Enhanced dissolution and absorption of poorly water-soluble drugs	● HP-β-CyD, SBE-β-CyD ● Methylated β-CyD ● Branched β-CyD
Prolonged release	Sustained release of water-soluble drugs	● Ethylated β-CyD ● Per-*O*-acylated β-CyD
Delayed release	pH-dependent (enteric) release of unstable drug or stomach irritating drug	● CME-β-CyD
Modified release	More balanced bioavailability with prolonged therapeutic effect	● Simultaneous use of CyDs and pharmaceutical excipients
Site-specific release	Colonic delivery Gene delivery	● Drug/CyD conjugate ● Dendrimer/CyD conjugate

work as prolonged-release carriers of water-soluble drugs [9]. Among the various acylated CyDs, per-*O*-butanoyl-β-CyD (TB-β-CyD) has the prominent retarding effect for water-soluble drugs, owing to the mucoadhesive property and appropriate hydrophobicity that differ from those of other derivatives having shorter or longer chains. As shown in Figure 2, combined use of per-*O*-acetyl-β-CyD (TA-β-CyD) and per-*O*-octanoyl-β-CyD (TO-β-CyD) gave a constant plasma level (20–40 ng/ml) of diltiazem for more than 48 h after oral administration to dogs. The longer acyl side chains, however, severely interfere with the ability of peracylated β-CyDs to form inclusion complexes [12]. The gel forming property of 2-hydroxypropyl-β-CyD (HP-β-CyD) is also useful to design the prolonged release of water-soluble drugs. For example, the release rate of metoprolol from the ternary metoprolol/HP-β-CyD/ethylcellulose (30/10)/60%w/v) tablet was barely influenced by pH of media, paddle rotation rate and viscosity of solutions and storage condition of the tablet [13]. A double layer tablet consisting of a fast-releasing fraction of nifedipine/HP-β-CyD complex and a slow-releasing fraction of nifedipine/hydroxypropylcellulose (HPC) dispersion gave prolonged plasma drug levels without decrease in bioavailability [14]. Moreover, sulfobutyl ether β-CyD (SBE7-β-CyD) can serve as both a solubility modulating and an osmotic pumping agent for the controlled-porosity osmotic pump tablets, from which

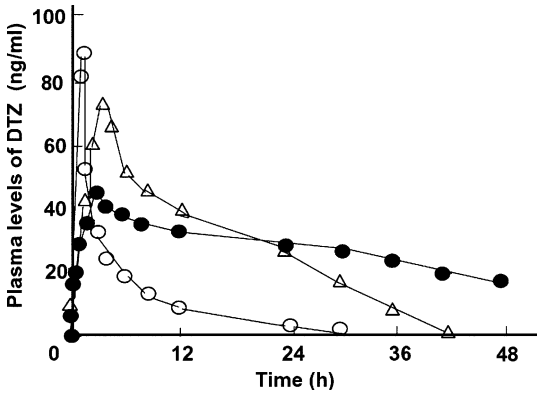


Figure 2. Plasma concentrations profiles of diltiazem (DTZ) following oral administration of tablets containing DTZ alone (○), DTZ/TA-β-CyD (Δ) in molar ratio of 1:2 and DTZ/TA-β-CyD/TO-β-CyD (●) in molar ratio of 1:2:0.5 systems (equivalent to 30 mg/body DTZ) in dogs [9]. Each point represents the mean ± S.E. of 3 dogs.

the release rate of both highly and poorly water-soluble drugs can be controlled precisely [15]. The ordinary delayed release profile can be obtained by the use of enteric type CyDs such as CME-β-CyD. The combined use of CyD complex and CyD conjugate will be useful for designing various kinds of time-controlled type oral drug delivery preparations [2]. The release of drug from the drug/CyD conjugate after oral administration shows a typical delayed-release behavior. Therefore, when the CyD conjugates are combined with other different release preparations, we can obtain more advanced and optimized drug release system, securing balanced oral bioavailability, and prominent therapeutic efficacy. For example, a repeated-release preparation may be designed by combining the CyD conjugate with a fast-releasing fraction, while a combined preparation of the conjugate with a slow-releasing fraction may provide a prolonged-release preparation. These modified-releases by means of the combination were demonstrated using the ketoprofen/α-CyD conjugate [16]. The co-administration of the CyD conjugate and the fast-dissolving ketoprofen/HP-β-CyD complex gave a typical repeated-release profile; i.e. double peaks were observed at about 1–2 h and 8–12 h in plasma drug levels after oral administration to rats (Figure 3). On the other hand, the co-administration of the conjugate and the slow-releasing ketoprofen/ethylcellulose solid dispersion gave a typical sustained-release profile; i.e. a constant plasma level was maintained for at least 24 h. These repeated and long circulating release patterns in plasma drug levels after oral administration were clearly reflected in the anti-inflammatory effect using rat with carageenan-induced acute edema in rat paw.

Since pharmaceutical preparations are usually composed of considerable amounts of pharmaceutical excipients and additives to maintain the efficacy and safety of the drug molecules, suitable combination of the CyD complex and the third component can markedly extend the actions of CyD for the design of advanced drug release formulations.

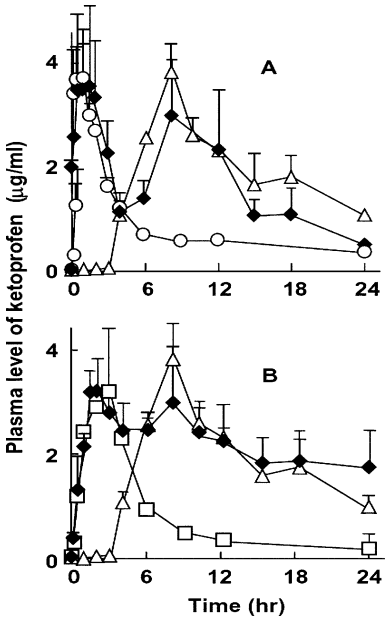


Figure 3. Plasma levels of ketoprofen after oral administration of combined preparations to rats [16]. (A) (Ketoprofen/α-CyD conjugate)/HP-β-CyD complex system: HP-β-CyD complex (○, equivalent to 2 mg/kg the drug), α-CyD conjugate (Δ, equivalent to 5 mg/kg the drug), and the combined system of conjugate and complex (♦, containing the equivalent amounts of the drug). (B) (Ketoprofen/α-CyD conjugate)/ethylcellulose solid dispersion system: dispersion (□, equivalent to 6 mg/kg the drug), α-CyD conjugate (Δ, equivalent to 5 mg/kg the drug), and the combined system of conjugate and dispersion (♦, containing the equivalent amounts of the drug). The drugs were administered as powder filled in capsules. Each point represents the mean of ± S.E. of 3–4 experiments.

Site-specific drug delivery

The inclusion equilibrium is sometimes disadvantageous when drug targeting is to be attempted, because the complex dissociates before it reaches the organ or tissues to which it is to be delivered. One of the methods to prevent the dissociation is to bind a drug covalently to CyD. CyDs are known to be barely capable of being hydrolyzed and only slightly absorbed in passage through the stomach and small intestine; however, they are fermented to small saccharides by colonic microflora and thus absorbed as maltose or glucose in the large intestine. Such biological property of CyDs is useful as a source of site-specific delivery of drugs to colon [2]. We have designed CyD conjugates of nonsteroidal anti-inflammatory drug, biphenylylacetic acid and ketoprofen, a short-chain fatty acid, *n*-butylic acid, a steroidal drug, prednisolone, and an anti-cancer drug, 5-fluorouracil, anticipating new candidates for colon-specific delivery prodrugs [2]. The drug molecules were selectively conjugated onto the primary or secondary hydroxyl groups of CyDs through an ester- or an amide-linkage, respectively, and their physicochemical properties and drug release behavior were investigated. The prednisolone/α-CyD ester conjugate (Figure 4), for example, was subject to the ring-opening of α-CyD followed by hydrolysis to the maltose and triose

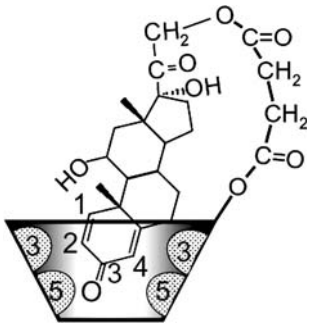


Figure 4. Proposed self-inclusion mode of prednisolone/α-CyD ester conjugates, estimated by NMR analysis [17].

conjugates [17]. The ester bond of small saccharide conjugates was subsequently hydrolyzed to form prednisolone which is absorbable from the cecum and colon. The anti-inflammatory effect and adverse effect of prednisolone/α-CyD conjugate were evaluated using inflammatory bowel disease (IBD) model rats prepared by administration of trinitrobenzenesulfonic acid [18]. The anti-inflammatory effect of prednisolone/α-CyD conjugate was comparable to that of drug alone, but the thymus/body weight ratio, a typical index of systemic side-effect in steroid therapy, was significantly reduced, probably due to the low plasma drug levels. Therefore, the prednisolone/CyD conjugate could be particularly useful for colon-specific delivery, owing to the alleviation of systemic side effects of prednisolone, while maintaining the anti-inflammatory effect. The present CyD prodrug approach can provide a versatile means for constructions of not only colon-specific delivery systems but also site-specific drug release system, including gene delivery.

Davis and co-workers [19] have reported a number of uses of β-CyD-containing polymers with adamantine–PEG or adamantine–PEG–transferrin for gene transfer as well as DNAzyme transfer. We have recently demonstrated that Starburst PAMAM dendrimer (generation 2 or 3) conjugate with α-CyD (α-CDE conjugates) in the molar ratio of 1:1 can be utilized as a novel non-viral vector for gene and siRNA delivery *in vitro* and *in vivo* [20]. The conjugate of the dendrimer with α-CyD (α-CDE conjugate, Figure 5) revealed a 100-fold increase in the activity, compared to that with plasmid DNA complexes with dendrimer. Next, we have revealed that of the three generations (G2, G3 and G4) of dendrimers in α-CDE conjugates, α-CDE conjugate (G3) had the most potent gene transfer activity. Furthermore, α-CDE conjugate (G3) with the average degree of substitution (d.s.) of 2.4 was found to demonstrate the greatest gene transfer activity, compared with TransFast™ and Lipofection™. In addition, we are designing a cell-specific gene delivery system using the α-CDE conjugates bearing various sugar moieties, such as the dendrimer (G2) conjugate with α-CyD (α-CDE conjugate (G2)) bearing mannose (Man-α-CDE conjugates) with the various d.s. of the mannose moiety (DSM) [21]. As a result, Man-α-CDE conjugates (DSM 3.3 and 4.9) were found to have much higher gene transfer activity than dendrimer, α-CDE conjugate and Man-α-CDE conjugates (DSM 1.1 and 8.3) in various cells, which are independent on the expression of cell-surface mannose receptors. Furthermore, the α-CDE conjugates bearing galactose with a spacer between dendrimer and galactose residues were found to have hepatocyte-specific gene transfer ability *in vitro*, suggesting the potential use of sugar-bearing α-CDE conjugates as a non-viral vector. These *in vitro* and *in vivo* results highlight the potential use of CyDs, CyD conjugates and CyD polymers for gene, antisense and siRNA therapies.

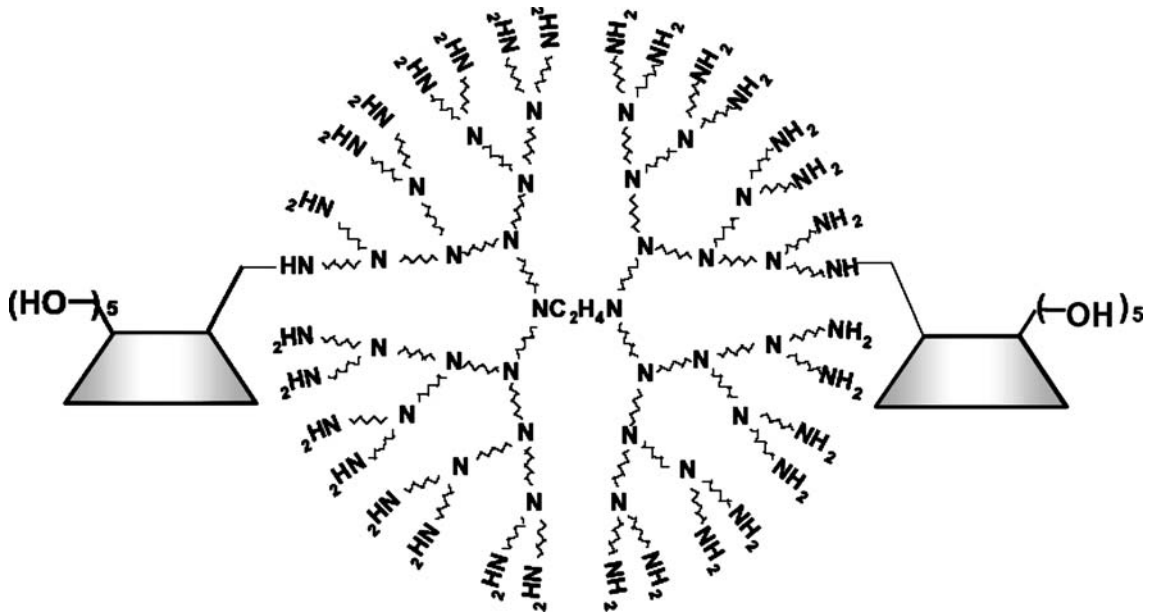


Figure 5. α-CyD/polyamidoamine dendrimer conjugate as non-viral gene transfer vector [20].

Table 3. CyD-based drug delivery systems

Use of CyD complex
● Hydrophilic CyDs
Improvement of solubility and oral bioavailability of poorly water-soluble drugs
Control of polymorphic transition and crystallization of drugs
● Hydrophobic CyDs
Sustained-release of water-soluble drugs
● Amphiphatic CyDs
Improvement of dermal/mucosal absorption of drugs
Stabilization of peptide/protein drugs
Use of CyDs conjugate
● Drugs/CyD conjugate: Colonic delivery, Time controlled release
● Cationic polymer/CyD conjugate: Gene delivery
Combined use of CyD with pharmaceutical excipients
● CyD/drug complex: Control of equilibrium, Competitive inclusion
● CyD/drug complex or conjugate with liposomes: Long circulation at targeting site

Conclusion

A number of bioadaptable CyD derivatives and polymers have been designed and evaluated for practical uses in pharmaceutical field in the form of complex or conjugate. Owing to the increasingly globalized nature of the CyD-related science and technology, development of the CyD-based pharmaceutical formulation is also rapidly progressing. The future should see a number of commercial products using various CyD-based advanced drug formulations (Table 3).

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