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Cyclodextrin-based controlled drug release system

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Abstract

Because of their bioadaptability and multi-functional characteristics, cyclodextrins (CDs) are capable of alleviating the undesirable properties of drug molecules in various routes of administration through the formation of inclusion complexes. This article outlines the current application of natural and chemically modified CDs in the design of advanced dosage forms. In an oral drug delivery system (DDS), the hydrophilic and ionizable CDs can serve as potent drug carriers in the immediate release- and delayed release-formulations, respectively, while the release rate of water-soluble drugs can be retarded by hydrophobic CDs. Since CDs are able to extend the function of pharmaceutical additives, the combination of molecular encapsulation with other carrier materials will become effective and a valuable tool in the improvement of drug formulation. Moreover, the most desirable attribute for the drug carrier is its ability to deliver a drug to a targeted site; conjugates of a drug with CDs can be a versatile means of constructing a new class of colon-targeting prodrugs. On the basis of this knowledge, the advantages and limitations of CDs in DDS are addressed.

Keywords: Cyclodextrin; Inclusion complex; Drug carrier; Oral drug delivery; Controlled release; Drug–cyclodextrin conjugate; Colon-targeting

Contents

1. Introduction

From the viewpoint of the optimization of the pharmacotherapy, drug release should be controlled

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in accordance with the therapeutic purpose and the pharmacological properties of active substances. There has been growing interest in developing rate- or time-controlled oral preparations, because appropriate drug release from dosage forms is of critical importance in realizing their therapeutic efficacy [1,2]. In order to design more advanced dosage forms, various kinds of carrier materials are being developed to deliver the necessary amount of drug to the targeted site for a necessary period of time, both efficiently and precisely [3]. Cyclodextrins (CDs) are potential candidates for such a role, because they can be used either for complexation or as functional carrier materials in pharmaceutical formulations [4–8]. One of the important characteristics of CDs is that they form inclusion complexes both in solution and in the solid state, in which each guest molecule is surrounded by the hydrophobic environment of the CD cavity. This can lead to alteration of physical, chemical and biological properties of guest molecules, and can eventually have considerable pharmaceutical potential. Recently, a number of CD derivatives and CD polymers have been prepared to obtain better inclusion abilities than found with parent CDs [9,10] (Table 1). β-CD, the most common natural CD, has 21 hydroxyl groups, that is, seven primary and 14 secondary hydroxyls (Fig. 1). All of these hydroxyl groups are available as starting points for structural modifications, and various functional groups have been introduced into the macrocyclic ring. Then the natural and chemically modified CDs have been extensively utilized to improve various drug properties, such as solubility, dissolution rate, stability or bioavailability [11–17]. The works pub-

Table 1
Pharmaceutically useful β-CD derivatives[a]

Derivative	Position of substituent	Substituent
Hydrophilic derivatives		
Methylated β-CD	2,6-; 2,3,6-	-O-CH_3
Hydroxyalkylated β-CD	Random	-O-CH_2-CH(OH)-CH_3
Branched β-CD	6-	-Glucosyl: -maltosyl
Hydrophobic derivatives		
Ethylated β-CD	2,6-; 2,3,6-	-O-C_2H_5
Peracylated β-CD	2,3,6-	-O-$CO(CH_2)_n$-CH_3
Ionizable derivatives		
Carboxyalkyl β-CD	Random	-O-$(CH_2)_n$-COONa
Carboxymethyl; ethyl	2,6-; 3-	-O-CH_2COONa; -O-C_2H_5
Sulfates	Random	-O-SO_3Na
Alkylsulfonates	Random	-O-$(CH_2)_n$-SO_3Na

[a]Obtained by substitution of the OH groups located on the edge of the CD ring.

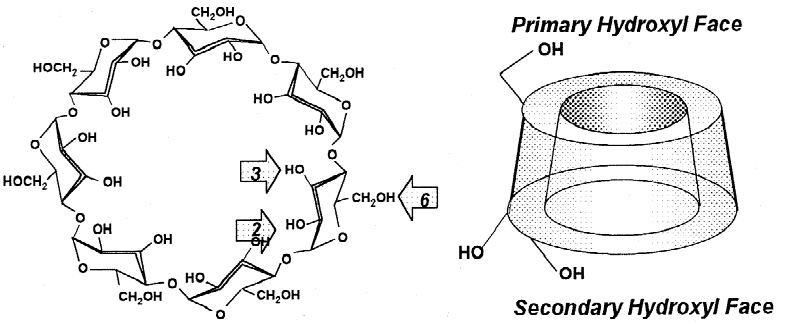


Fig. 1. Hydroxyls located on the edge of the β-CD ring.

lished thus far apparently indicate that the multifunctional characteristics of CDs may allow the rational design of drug formulation and that the combination of molecular encapsulation with other carrier systems will become a very effective and valuable method for the development of new drug delivery systems [18–21]. Among the chemically modified CDs, the hydrophilic [22–27] or ionizable CDs [28–31] will enhance drug absorption, while hydrophobic CDs may have broad applicability, and could serve as novel slow-release carriers of water-soluble drugs, including peptide and protein drugs [32–38]. Moreover, the biodegradation property of CD [39–41] is particularly useful as a colon-targeting carrier, and the CD prodrugs will serve as a source of site-specific delivery of drugs to the colon [42]. Thus, the objective of this contribution is to focus on the potential use of natural and chemically modified CDs as high performance drug carriers in the development of advanced dosage forms with emphasis on the latest applications of CDs in site-specific delivery.

2. Controlled release in oral drug delivery systems

Fig. 2 shows typical drug release–time profiles after oral administration. The plasma drug levels–time profiles can be mainly classified into two categories, i.e. the rate-controlled type and the time-controlled type (delayed release type). The rate-controlled type is further classified into three types, i.e. immediate release, prolonged release and modified release types. On the basis of this knowledge, various CD derivatives have been used in order to modify drug release in oral preparations (Table 2).

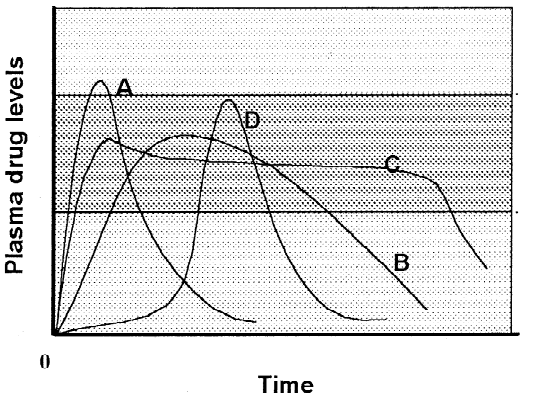


Fig. 2. Typical drug release profiles following oral administration. (A) Immediate release; (B) Prolonged release; (C) Modified release; (D) Delayed release.

The hydrophilic and hydrophobic CDs are useful for the immediate release and the prolonged release type formulations, respectively. The delayed release type formulation can be obtained by the use of *O*-carboxymethyl-*O*-ethyl–β-CD (CME-β-CD). Moreover, a combination of CDs and other carrier materials is useful for optimizing the release rate of drugs. Typical examples of the applications of CD derivatives in modified release and prolonged release formulations will be described next.

Table 2
Modification of the drug release site and/or time profile by CDs

Release pattern	Aim	Use of CD
Immediate-release	Enhanced dissolution and absorption of poorly water-soluble drugs	HP-β-CD, DM-β-CD, SB-β-CDs, Branched β-CDs
Prolonged-release	Sustained release of water-soluble drugs	Ethylated β-CDs, Acylated β-CDs
Modified-release	More balanced oral bioavailability with prolonged therapeutic effects	Simultaneous use of different CDs and/or other excipients
Delayed-release	(Enteric)	
pH-dependent release	Acid protection of drugs	CME-β-CD
Site-specific release	Colon-targeting	Drug–CD conjugate

2.1. Immediate release

Immediate release formulations of analgesics, antipyretics, coronary vasodilators, etc. are particularly useful in emergency situations. Since the dissolution rate of the poorly water-soluble drugs is mainly responsible for both the rate and extent of oral bioavailability of drugs, various hydrophilic materials are used to attain an immediate release formulation. The hydrophilic CDs have been extensively used to enhance the oral bioavailability of steroids, cardiac glycosides, nonsteroidal antiinflammatory drugs, barbiturates, antiepileptics, benzodiazepines, antidiabetics, vasodilators, etc. [4–11]. These improvements are mainly ascribable to the increase in solubility and wettability of drugs through the formation of inclusion complexes. The commercial viability of CD-based oral formulations has been established with the marketing of more than ten products [17].

The rate and extent of oral bioavailability of a poorly water-soluble drug from its CD complex can be optimized by adjusting various factors that affect the dissociation equilibrium of the complex both in the formulation and in the biophase in which the complex is administered [10,16]. Only a free form of the drug, which is in equilibrium with the complexed form of the drug in solution, is capable of penetrating lipophilic barriers consisting of either mucosal epithelia or stratified cell layers and eventually entering the systemic circulation. In general, maximum absorption enhancement is obtained when just enough CD is used to solubilize all of the drug in solution. Further addition of CD to the drug solution decreases the free fraction of the drug and, hence, reduces the drug's bioavailability. Practical formulations usually contain a large quantity of pharmaceutical excipients, which may compete with the drug for the CD cavity. Such competition may also occur with endogenous substances that exist at the absorption site. The displacement of the drug from the CD cavity by exogenous and endogenous substances at the absorption site is responsible for acceleration of drug absorption [43,44]. For instance, the overall process of drug absorption from a solid complex in the presence of competing agent is shown in Fig. 3 [12], where k_d is the dissolution rate constant, K_c is the stability constant of the complex of the drug with the CD, K_i is the stability constant of the complex of the competing agent with the CD and k_a is the absorption rate constant of the drug. A high dissolution rate and relatively stable complexes ($K_i > K_c$) favor a free drug that is readily available for absorption. By contrast, a free CD after the dissociation of the complex removes some components from the membrane surface, thereby modifying the transport properties of the membranes and facilitating drug absorption, especially for water-

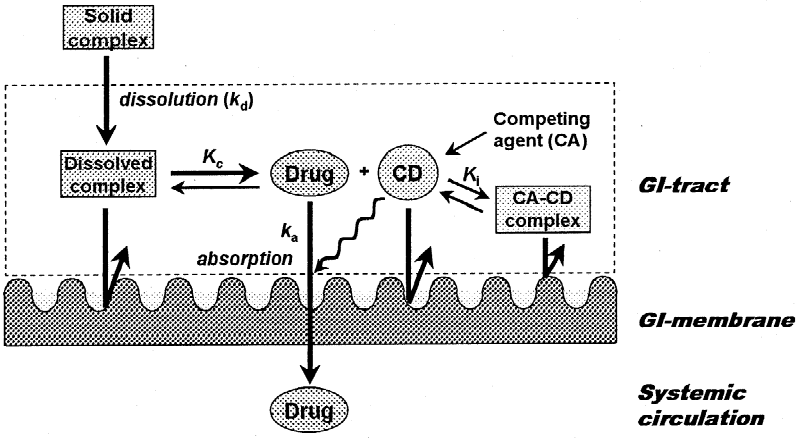


Fig. 3. Overall process of drug absorption from an inclusion complex following dissolution and dissociation in the gastrointestinal tract. Modified from ref. [12]; k_d, dissolution rate constant of drug–CD complex; K_c, stability constant of drug–CD complex; K_i, stability constant of competing agent–CD complex; k_a, absorption rate constant of drug.

soluble drugs. Thus, attention should be directed towards the dissociation equilibrium and stoichiometry of the complex in both body fluids and pharmaceutical formulations.

As shown in Fig. 3, CDs are supposed to act only as carrier materials and help to transport the drug through an aqueous medium to the lipophilic absorption surface in the gastrointestinal tracts. Therefore, such applications have been successful when the rate-limiting step in drug absorption is dissolution of the drug itself and not absorption across the gastrointestinal tract. Recent studies have shown that complexation of β-CD with imidazole antifungal agents, such as ketoconazole and econazole, provided an enhancement of oral bioavailability [45,46]. Other studies have demonstrated that a combination of α-CD with citric acid is effective in preventing gel formation of cefotiam hexetil hydrochloride, an orally active antibacterial agent, increasing the dissolution rate and improving its oral bioavailability [47]. The stabilizing effect of CDs on labile drugs is also responsible for the improvement of oral bioavailability. For example, a γ-CD complex was found to decrease acid hydrolysis of cardiac glycosides and, hence, to improve the oral absorption of digoxin in dogs [48].

Recently, highly hydrophilic CD derivatives, such as 2-hydroxypropyl–β-CD (HP-β-CD) [49], maltosyl–β-CD (G_2-β-CD) [50], and sulfobutylether–β-CDs (SBE-β-CDs) [51] have been used to obtain an immediate release formulation that is readily dissolved in the gastrointestinal tract, enhancing the oral bioavailability of poorly water-soluble drugs. HP-β-CD is utilized to modify the physical properties of drugs in the solid state, such as particle size, the polymorphic transition and the conversion from the crystalline to an amorphous or glassy state [52,53]. For example, the rapidly dissolving forms of metastable and glassy states of nifedipine can be obtained by cooling the melts of a 1:1 physical mixture of drug–HP-β-CD matrices [54].

Rapidly dissolving complexes of drugs with hydrophilic CDs are well suited for sublingual or buccal administration. This type of drug entry not only gives a rapid rise in systemic drug concentrations but also avoids intestinal and hepatic first-pass metabolism of the drug. Other illuminating results were obtained for the sublingual administration of tablets containing complexes of steroids with CDs [55–60]. HP-β-CD and β-CD polymers supported the absorption of testosterone from the oral cavity and not from the gastrointestinal tract; these solubilizers neither enter nor damage the oral tissues [61]. Inherently, the blood level of endogenous testosterone rises a few times a day in episodes that last approximately 1 h. Such pulsatile release of testosterone can be imitated by the sublingual administration of its HP-β-CD complex, giving the desired pharmacological effects [57]. This formulation may be especially suitable for treatment of boys with delayed puberty and older men with an androgen deficiency [59].

Another approach is the use of amphiphilic CDs, such as β- or γ-CD esterified on the secondary hydroxyl groups by alkyl chains (from C_6 to C_{14}). They are capable of forming self-assembled nanospheres that can be loaded with high amounts of poorly water-soluble drugs, such as indomethacin and progesterone. The drug, being molecularly dispersed in the nanospheres, is very rapidly released in aqueous medium, allowing for the administration of poorly water-soluble drugs that can be bioavailable rapidly [62].

2.2. Delayed release

An enteric preparation can be classified as time-controlled release, since the drug is preferentially released in the intestinal tract. Hydrophobic excipients having a weak acidic group are preferable because they are less soluble in water at low pH, but soluble in neutral and alkaline regions due to the ionization of the acidic group. Under the control of this pH dependence, the delayed release dosage form, which passes from the stomach into the higher pH environment of the upper small intestine, would experience increased drug release. For this purpose, CME-β-CD was developed to exhibit pH-dependent solubility for use in selective dissolution of the drug–CD complex [28]. As shown in Fig. 4, CME-β-CD displays limited solubility under acidic conditions, such as those found in the stomach, with the complex solubility increasing as the pH passes through the pK_a (3.7) of the CME-β-CD. CME-β-CD complexes have been used in in-vitro and in-vivo

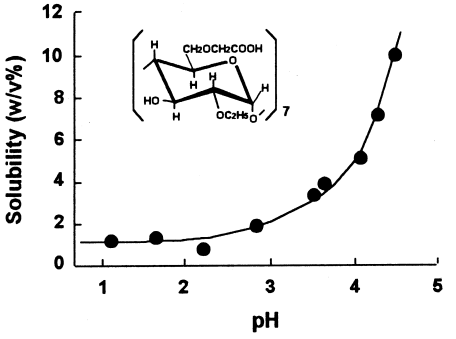


Fig. 4. Solubility of CME-β-CD as a function of pH in aqueous solution at 25°C. (see ref. [28]).

studies with diltiazem, a calcium-channel antagonist, and molsidomine, a peripheral vasodilator. The diltiazem studies were carried out in gastric acidity-controlled fasting dogs with the gastric pH being controlled to less than two and greater than six. Diltiazem absorption was slower at high gastric acidity (t_{max}, 4.0±0.5 h) than at low gastric acidity (t_{max} = 2.3±0.2 h) (Fig. 5). The in-vitro release data measured using a pH changeable dissolution apparatus were in good agreement with the in-vivo data [63]. Molsidomine absorption from tablets containing CME-β-CD was studied in gastric acidity-controlled dogs in the fasted and fed states. Under high gastric acidity, molsidomine absorption was significantly retarded relative to that found under low gastric acidity conditions. The delayed absorption

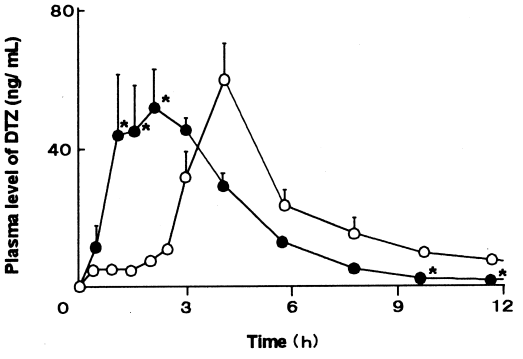


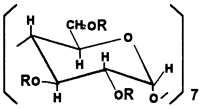
Fig. 5. Plasma levels of diltiazem after the oral administration of tablets containing CME-β-CD complex (equivalent to diltiazem at 30 mg/body) in gastric acidity-controlled dogs (see ref. [63]); ○, high gastric acidity dogs; ●, low gastric acidity dogs. Each value represents the mean±standard error of four dogs. *, $p < 0.05$ vs. high gastric acidity dogs.

effect under high gastric acidity was more pronounced under fasted conditions. As in the diltiazem studies, a high degree of correlation was noted between the in-vivo studies and the in-vitro release measured with the pH-changeable dissolution apparatus [64].

2.3. Prolonged release

Most of the slow-release preparations have been aimed at achieving zero-order or pH-independent release of drugs to provide a constant blood level for a long period of time. This kind of formulation has many advantages, such as reducing the frequency of dosing, prolonging the drug's efficacy and avoiding the toxicity associated with the administration of a simple plain tablet. For this purpose, hydrophobic CDs, such as alkylated and acylated derivatives, are useful as slow-release carriers for water-soluble drugs. Among the alkylated CDs, 2,6-di-*O*-ethyl–β-CD (DE-β-CD) and 2,3,6-tri-*O*-ethyl–β-CD (TE-β-CD) were the first slow-release carriers to be used in conjunction with diltiazem [33,65] and isosorbide dinitrate [66] following oral administration of the hydrophobic complexes to dogs. On the other hand, the peracylated CDs with medium alkyl chain lengths (C_4–C_6) are particularly useful as novel hydrophobic carriers (Table 3), because of their multifunctional and bioadaptable properties [32,67]. They have broad applicability in various routes of administration: for example, the bioadhesive property of 2,3,6-tri-*O*-butyryl–β-CD (TB-β-CD) (C_4) can be used in oral and transmucosal formulations, while the film-forming property of 2,3,6-tri-*O*-valeryl–β-CD (TV-β-CD) (C_5) is useful in transdermal preparations [68]. In oral applications, molsidomine was used to design a sustained release formulation, because this drug is water-soluble and has a short biological half-life. The release rate of molsidomine was markedly retarded by complexation with peracylated β-CDs, in decreasing order of their solubility, in particular, by those longer than TB-β-CD [69]. When the complexes were administered orally to beagle dogs, TB-β-CD suppressed a peak plasma level of molsidomine and maintained a sufficient drug level for long periods, while the single use of other derivatives having shorter or longer chains than TB-β-CD proved to be insuffi-

Table 3

Some physicochemical properties of acylated β-CDs 

Compound	*R*	Melting point (°C)	$[M]_D^a$	Solubility[b] (mg/dl)
β-CD	H	280	+ 1850[d]	119.0
TA-; peracetyl–β-CD	$COCH_3$	201–202	+ 2522	823.0
TP-; perpropionyl–β-CD	COC_2H_5	168–169	+ 2450	423.5
TB-; perbutyryl–β-CD	COC_3H_7	126–127	+ 2607	219.8
TV-; pervaleryl–β-CD	COC_4H_9	54–56	+ 2640	283.0
TH-; perhexanoyl–β-CD	COC_5H_{11}	– [c]	+ 2620	3.7
TO-; peroctanoyl–β-CD	COC_7H_{15}	– [c]	+ 2763	– [e]
TD-; perdecanoyl–β-CD	COC_9H_{19}	– [c]	+ 2668	– [e]
TL-; perlauroyl–β-CD	$COC_{11}H_{23}$	– [c]	+ 2829	– [e]

[a]In chloroform at 25°C; [b]In 80% (v/v) ethanol–water at 25°C; [c]Oily substance; [d]In water; [e]Could not be determined because of the low solubility.

cient (Fig. 6). Prolonged maintenance (at least 24 h) of higher and constant levels of salbutamol, a bronchodilator, in plasma was also obtained after oral administration of the TB-β-CD complex in dogs, where the plasma level of the major metabolite, salbutamol glucuronide, was significantly lower than that found after administration of the drug alone [70]. This indicates that TB-β-CD may be a useful carrier for orally administered water-soluble drugs, especially for drugs that are metabolized in the gastrointestinal tract. The superior sustaining effect exhibited with TB-β-CD may be a result of both increased hydrophobicity and mucoadhesive properties. Similarly, nanospheres formed by amphiphilic CDs such as 2,3-di-*O*-hexanoyl–β-CD (DH-β-CD) may have bioadhesive effects on gastrointestinal mucosa [62].

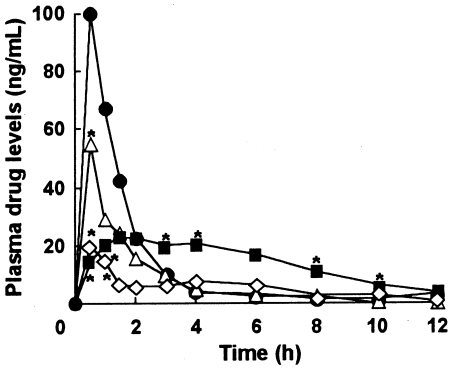


Fig. 6. Plasma levels of molsidomine after oral administration of capsules containing molsidomine or its peracylated β-CD complex (equivalent to 10 mg molsidomine) in dogs (see ref. [69]). ●, drug alone; △, TA-β-CD complex; ■, TB-β-CD complex; ◇, TH-β-CD complex. Each value represents the mean±standard error of three–six dogs. *, $p < 0.05$ vs. drug alone.

The combined use of short and long chain peracylated β-CDs in an appropriate molar ratio is also effective in controlling the release rate of water-soluble drugs [71]. For example, the release rate of diltiazem decreased with increasing hydrophobicities of the carrier materials (Fig. 7). The change in release rate of the drug from the hydrophobic carriers was clearly reflected in the drug levels found in blood after oral administration of the tablets to dogs. Although the drug–2,3,6-tri-*O*-acetyl–β-CD (TA-β-CD) (C_1) system, at a molar ratio of 1:2, maintained plasma drug levels of over 30 ng/ml for at least 24 h, a combination of TA-β-CD and 2,3,6-tri-*O*-octanoyl–β-CD (TO-β-CD) (C_8) gave a constant plasma drug level (20–40 ng/ml) for more than 48 h, with a significant increase in the extent of bioavailability.

2.4. Modified release

The conventional formulation of nifedipine, a typical calcium-channel antagonist, must be given either twice or three times daily, because of the short elimination half-life due to the considerable first-pass metabolism. Moreover, it has some pharmaceutical

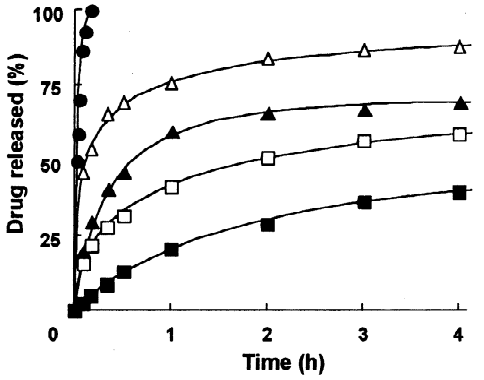


Fig. 7. Release profiles of diltiazem from tablets containing diltiazem, diltiazem–TAβ-CD and diltiazem–TA-β-CD–TO-β-CD systems (equivalent to 6 mg diltiazem) in water at 37°C, measured by the rotating basket method at 60 rpm (see ref. [71]). ●, diltiazem alone; △, diltiazem–TA-β-CD complex (molar ratio of 1:1); ▲, diltiazem–TA-β-CD complex (molar ratio of 1:2); □, diltiazem–TA-β-CD–TO-β-CD complex (molar ratio of 1:2:0.25); ■, diltiazem–TA-β-CD–TO-β-CD complex (molar ratio of 1:2:0.5).

problems, such as low oral bioavailability due to poor aqueous solubility, and a decrease in its dissolution rate during storage (due to crystal-growth) [72]. Therefore, the release rate of nifedipine must be modified in order to obtain a more balanced oral bioavailability with prolonged therapeutic effect. Wang et al. [73–75] have recently developed a double-layer tablet, using HP-β-CD and pharmaceutical excipients, and evaluated the drug's release behavior. In these studies, an amorphous nifedipine powder, prepared by spray-drying with HP-β-CD and a non-ionic detergent, HCO-60®, was employed as a fast-release portion to attain initial rapid dissolution and to prevent crystal growth during storage. Hydroxypropylcelluloses (HPCs) with different viscosity grades were employed as a slow-release portion to provide an appropriate level of sustained release of poorly water-soluble nifedipine from viscous matrices. Then, an optimal formulation of the double-layer tablet was surveyed by changing the mixing ratio of the fast-release portion and the slow-release portion (Fig. 8).

The in-vitro release rate of nifedipine from the double-layer tablet was little affected by the pH of the medium and the rotation speed of the paddle even after long-term storage under accelerated conditions (60°C, 75% relative humidity) [74]. Among the seven formulations tested, the double-layer tablet (F_3), consisting of HP-β-CD with 3% HCO-60®/[hydroxypropylcellulose L (low viscosity grade)–hydroxypropylcellulose M (medium viscosity grade)] in a weight ratio of 1/(1.5:1.5) was selected as the most appropriate one, because it elicited a prominent retarding effect with superior oral bioavailability compared with those of a commercially available slow-release product [75] (Fig. 9). These facts suggest that a combination of HP-β-CD, HCO-60® and hydroxypropylcelluloses serves as a modified-release carrier of nifedipine, and can be applied to

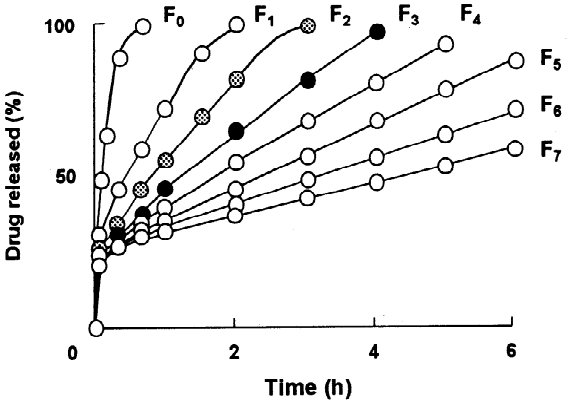


Fig. 8. Release profiles of nifedipine (5 mg) from plain tablet (F_0) and seven double-layer tablets (F_1–F_7) in water at 37°C (see ref. [75]).

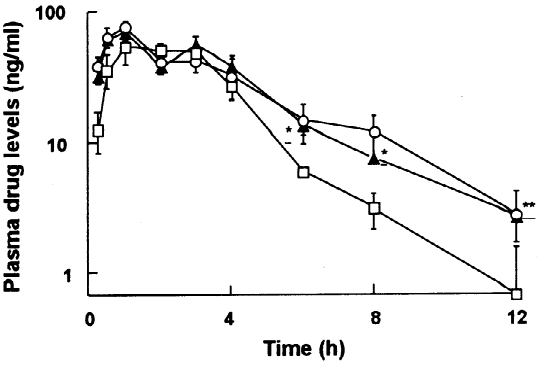


Fig. 9. Plasma levels of nifedipine after oral administration of tablets (20 mg as nifedipine) in dogs (see ref. [75]). ○, double-layer tablet (F_2); ▲, double-layer tablet (F_3); □, Adalat L-20. Each value represents the mean±standard error of four dogs.

other poorly water-soluble drugs with a short elimination half-life.

When the stomach is one of the important absorption sites, there should be no lag-time in drug release from the dosage form. This type of dosage form is required for loop diuretics such as furosemide and piretanide, in which the release of a certain amount of drug in the stomach is desired to give more balanced bioavailability. To attain efficient bioavailability for such acidic drugs, double-layer tableting was also useful, consisting of hydrophilic CDs as the fast-release portion and hydrophobic cellulose derivatives as the slow-release portion [76]. In the case of piretanide, a tablet consisting of a [DM-β-CD/(hydroxypropylcellulose/ethylcellulose)] system, at a weight ratio of 1/3(1/3) provided sufficient slow release of the drug over 8 h and over a wide pH region following initial rapid dissolution.

Poorly water-soluble drugs often demonstrate erratic oral bioavailability that is further exacerbated when attempts are made to develop controlled release dosage forms. A porosity-controlled osmotic pump tablet (OPT) is one coated with a semipermeable membrane containing leachable pore-forming materials. The idea was first developed formally by Zentner et al. [77]. In this system, drug, after dissolution, is released from the tablet by hydrostatic pressure through pores created by the dissolution of pore formers incorporated into the membrane. Hydrostatic pressure is created by an osmotic agent, e.g. the drug itself or a tablet component, after water is imbibed across the semipermeable membrane. This system is generally applicable for only highly water-soluble drugs. Because poorly water-soluble drugs dissolve slowly, it is not possible to completely deliver drugs with poor solubility from such devices and tablets in general. This problem can be overcome by adding $(SBE)_{7m}$–β-CD (there are about seven degrees of substitution of the sulfobutyl ether group on β-CD hydroxyls and these can act as both a solubilizer and as an osmotic agent) [78]. Recently, Okimoto et al. [79] developed a novel OPT for prednisolone using $(SBE)_{7m}$–β-CD, which acts as a solubilizer and an osmotic agent. Core tablets, in an OPT containing the drug and osmotic agents, were film-coated with cellulose acetate. In-vitro release studies revealed that prednisolone was completely released from OPTs with $(SBE)_{7m}$–β-CD, in contrast to those in HP-β-CD or sugars. The plasma drug profiles for OPTs following oral administration to dogs were retarded, with a decreased in-vitro rate. This technique has also been applied to chlorpromazine [80], a weak basic drug, which exhibits pH-dependent solubility and is poorly soluble at neutral pH.

3. Cyclodextrin-based site-specific drug delivery

Recently, intensive efforts have been made to design a system that is able to deliver drugs more efficiently to specific organs, tissues and cells, etc. CD complexes are in equilibrium with guest and host molecules in aqueous solution, with the degree of the dissociation being dependent on the magnitude of the stability constant of the complex. This property is a desirable quality, because the complex dissociates to give free CD and drug at the absorption site, and only the drug in free form enters into the systemic circulation. A typical example of this is the application of HP-β-CD to the chemical delivery system developed by Bodor [81], which will be described below. However, the inclusion equilibrium is sometimes disadvantageous when drug targeting is to be attempted, because the complex dissociates before it reaches the organs or tissues to which it is to be delivered. One of the methods to prevent dissociation is to bind a drug covalently to CDs. In this section, recent results on site-specific delivery using CDs are described.

3.1. CD–drug conjugates as colon-targeting prodrugs

Colon targeting is essentially classified as a delayed release with a fairly long lag-time (Fig. 10), because the time required to reach the colon after oral administration is expected to be about 8 h in man [82,83]. When a CD complex is given orally, it will readily dissociate in the gastrointestinal fluid, depending on the magnitude of the stability constant. This indicates that CD complexes are not suitable for colon-specific delivery due to release of the drug (because of dilution and/or competitive inclusion effects) before it reaches the colon. On the other hand, the physicochemical and biopharmaceutical

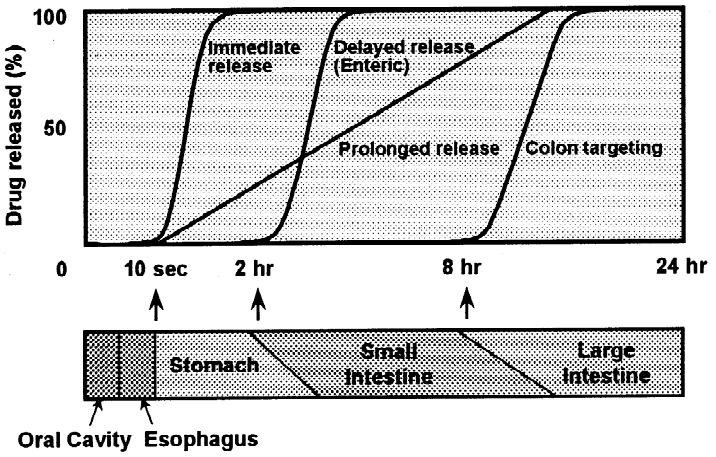


Fig. 10. Release profiles of drug from various dosage forms following oral administration.

properties of CD–drug conjugates, in which a drug is covalently bound to CD, must differ greatly from those of the inclusion complex. One of the advantages of CD–drug conjugates is that they can survive passage through the stomach and small intestine, however, drug release will be triggered by enzymatic degradation of CDs in the colon. Taking these factors into account, we [42,84] have designed amide- and ester-type conjugates of the antiinflammatory drug biphenylylacetic acid (BPAA) with three natural CDs, anticipating a new candidate for a colon-targeting prodrug (Fig. 11) [82,83].

Interestingly, the solubility of the CD-based prodrugs is related to the cavity size of CD. For example, the extremely low solubility of the β-CD conjugate was ascribed to the intermolecular association between the drug moiety and the neighboring CD cavity. On the other hand, the highest solubility

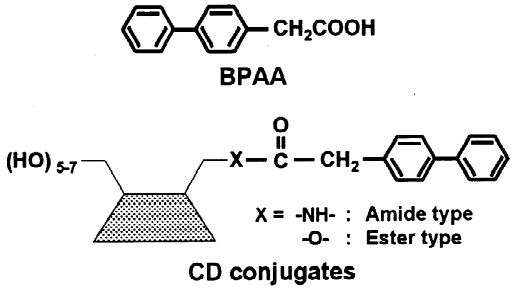


Fig. 11. Structures of biphenylylacetic acid (BPAA) and its cyclodextrin conjugates.

observed for the α-CD conjugate may be due to the fact that α-CD cavity is too small to accommodate the BPAA moiety. The release profiles of the drug after incubation of the ester conjugates in rat gastrointestinal tract contents, intestine and liver homogenates, and blood in isotonic buffer solutions, were then compared with those of ethyl biphenylyl acetate (EBA), a simple ethyl ester of BPAA. EBA was easily hydrolyzed in liver and gastrointestinal tract homogenates, and also in blood, however, it was stable enough in the cecal and colonic contents. In sharp contrast, the α- and γ-CD ester conjugates released BPAA significantly in cecal and colonic contents, while no appreciable drug release from the conjugates was observed on incubation with other contents or fluids. When the ester conjugates were incubated with rat cecal contents, α- and γ-CD conjugates produced BPAA quantitatively, while the β-CD conjugate released BPAA in only small amounts, despite the significant disappearance of the β-CD conjugate. Although the drug release patterns of the three CD conjugates are different from each other, the in-vitro data clearly suggest that ester conjugates are first subject to the ring-opening of CD by bacterial enzymes, to give the triose and maltose conjugates through to longer linear oligosaccharide conjugates. Thus, the ester linkage of the small saccharide–BPAA conjugates could be highly susceptible to hydrolysis. On the other hand, the amide conjugates hardly released the drug at all, despite fermentation to small saccharide–BPAA conjugates.

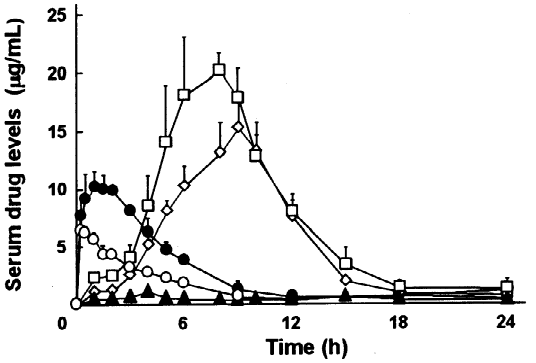


Fig. 12. Serum levels of BPAA after the oral administration of suspensions containing BPAA, β-CD complex or CD ester conjugates (equivalent to 10 mg/kg BPAA) in rats (see ref. [42]). ○, BPAA alone; ●, β-CD complex; ◇, α-CD ester conjugate; ▲, β-CD ester conjugate; □, γ-CD ester conjugate. Each value represents the mean±standard error of two–four rats.

Fig. 12 shows the serum levels of BPAA after oral administration of three ester conjugates, compared with the drug alone and a β-CD complex in rats. As expected, the fast-dissolving form of CD complex shows a rapid increase and decrease in the serum drug levels, compared with that of drug alone. In the case of β-CD conjugate, a slight increase in serum drug levels was observed, probably due to the slower drug release. However, the serum drug levels of the α- and γ-CD conjugates increased after a lag time of about 3 h, and reached maximum levels at about 9 and 8 h, respectively, accompanied by a significant increase in the extent of bioavailability. The extent of bioavailability for the α- and γ-CD conjugates were about four and five times larger, respectively, than that for BPAA alone. In-vivo studies further revealed that BPAA is released and absorbed in the cecum and colon after oral administration of the ester type conjugates in rats, which may consequently provide the long lag time. The antiinflammatory effects of the γ-CD ester conjugate and β-CD complex were evaluated using the model of carrageenin-induced acute edema in rat paw [85]. In the case of the β-CD complex, a rapid antiinflammatory response was observed, compared to that of BPAA alone, because the drug was mainly absorbed from the small intestine after fast dissolution of the complex. In sharp contrast, the γ-CD ester conjugate needed a fairly long lag time to exhibit the drug's activity, because BPAA was produced after it had reached the cecum and colon. On the basis of the above-mentioned results, the release mechanism of BPAA from its γ-CD prodrugs, as an example, in rat cecum and colon, could be as proposed in Fig. 13. In the case of ester-type conjugates, drug release is triggered by the ring-opening of CDs, which consequently provides site-specific drug delivery in the colon. On the other hand, the amide conjugates do not release the drug even in the cecum and colon, despite the ring-opening of CDs. The amide linkage of the small saccharide–drug conjugates may be resistant to the bacterial enzymes and poorly absorbable from the intestinal tract due to high hydrophilicity. Therefore, the ester-type conjugate is pre-

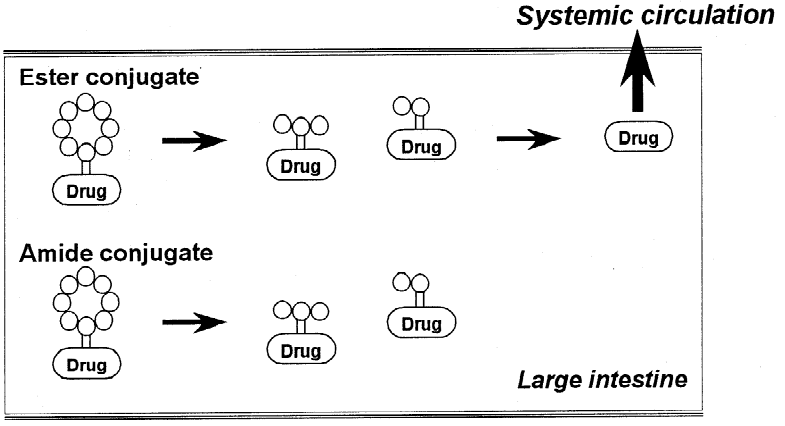


Fig. 13. Release mechanism of drug from γ-CD–drug conjugates in rat cecum and colon. Modified from ref. [85].

ferable as a delayed release-type prodrug that can release a parent drug selectively in the cecum and colon.

3.2. Brain targeting

When a drug is covalently coupled to 1-methyl-1,4-dihydronicotinic acid through an enzymatically labile linkage, its lipophilicity increases and allows selective delivery of drug molecules into the brain across the blood–brain barrier, which is characterized by the endothelial cells of cerebral capillaries that have tight continuous circumferential junctions, thus restricting the passage of polar drugs to the brain [86]. After entry into the brain, the dihydropyridine moiety is oxidized by oxidoreductase to 1-methyl-pyridinium cation. Thus, the polar drug–1-methylpyridinium derivative is trapped in the brain due to the presence of the blood–brain barrier. Subsequently, the parent drug is released from the prodrug by the action of other enzymes. This is an essential concept of Bodor's chemical delivery system and is applied to brain targeting of drugs such as steroids, antitumor agents and calcium-channel antagonists [87]. The problem, however, is that the prodrugs of the chemical delivery system are poorly water-soluble due to the presence of the lipophilic moiety. HB-β-CD solved this solubility problem by means of soluble complex formation, together with enhancing the chemical stability of the dihydronicotinic acid in aqueous solution. For example, the i.v. administration of estradiol chemical delivery systems (5 mg/kg) solubilized in 20% HB-β-CD produced a higher concentration of the prodrug in rat brain, which was almost the same as that produced by the administration of the prodrug (15 mg/kg) solution in dimethylsulfoxide [88]. It is apparent that HB-β-CD has an advantage over dimethylsulfoxide from a safety point of view. The improvement in chemical delivery systems using HB-β-CD was reported for testosterone [89], dexamethasone [90] and benzyl penicillin [91].

The specific delivery of potential neuropharmaceuticals to the brain is obstructed by the presence of the blood–brain barrier. One of the strategies to overcome this transport problem is to prepare prodrugs with high lipophilicity that pass through the blood–brain barrier. Unfortunately, the applications of CDs to brain targeting are few. One of the examples is the β-CD conjugates with δ-opioid receptor peptides, *N*-leucine-enkephalin and its cyclic analogue [*p*-I-Phe^4, D-Pen^2, D-Pen^5]enkephalin, where the carboxyl group of the C-terminal leucine was coupled with 6-amino-6-deoxy–β-CD and in the latter conjugate, all hydroxyl groups of CDs were further methylated to increase the lipophilicity [92,93]. Although the potency of the latter conjugate decreased in the receptor binding assay and in the in-vitro guinea pig intestine and mouse spermatic duct bioassay systems, it showed potent antinociceptive properties when given i.c.v and i.v. in the mouse tail flick test and exhibited no toxicity. Furthermore, it showed prolonged action in the bioassay. Although the detailed transport mechanism of these conjugates to the brain has not been fully elucidated, this methodology can be applied to other neuropharmaceuticals such as morphine. The β-CD conjugate with *N*-leucine-enkephalin is of interest, because it has a vacant cavity and can include a neurotropic drug, dothiepine. In general, the substituents introduced at primary hydroxyl groups of CDs, through a spacer of appropriate length, are self-included within the cavity. However, the enkephalin conjugate can accommodate other guest molecules, probably because the self-inclusion is restricted due to a steric hindrance. This inclusion property of conjugates may be useful from the viewpoint of drug formulation, since two different drugs can be incorporated into the CD molecule.

3.3. Specific cell targeting

β-Lactam antibiotics exert their lethal effect by inhibiting the synthesis of bacterial peptidoglycan, thereby disrupting the cell morphology and eventually resulting in cell lysis and death [94]. Since the antibacterial effect of β-lactam is affected by the permeability of the outer membrane of bacteria, it is important in the evaluation of drug efficacy to measure the diffusion rate of drugs across the membrane. However, the β-lactamase enzyme expressed at the cell surface interferes with this measurement. In order to solve this problem, a β-lactamase inhibitor that is water-soluble and could reach the cell membrane, but not penetrate it, must be prepared. Kurunaratne et al. [95] chose β-CD as a bulky moiety for the prevention of drug entry across channels in the bacterial outer membrane; thus, they

prepared β-CD conjugates linked to an antibiotic, methicilline, at the molecular terminus through spacers of different lengths (4.7–23.7 Å) [95]. The extent of inhibition of the surface β-lactamase of *P. Aeruginosa* in the presence of 10 mM of each of the conjugates was as follows: 6% using the conjugate with the spacer length of 12.0 Å, 20% using the 14.0 Å conjugate, 43% using the 16.8 Å conjugate, 77% using the 20.0 Å conjugate, 91% using the 22.6 Å conjugate, and 100% using the 23.7 Å conjugate. They concluded that the length of the spacer should be greater than 16 Å for optimum inhibition of β-lactamase in the outer membrane. Kim et al. [96] reported that the α-CD conjugate with antitumor sulfonylurea selectively blocks NADH oxidase activity at the external plasma membrane surface of HeLa cells.

Intercellular recognition events are fundamental to many biological processes in which oligosaccharides on cell surface glycoproteins or lectins have been responsible for cell–cell recognition and adhesion, etc. [97]. Therefore, CD derivatives bearing small saccharides may be useful as a carrier for transporting active drugs to sugar receptors such as lectins located on the cell surface. In accordance with this concept, several CD conjugates with mono- and disaccharides, such as glucose, galactose, mannose, fucose, etc., have been prepared and investigated for binding characteristics to sugar-specific receptors [98–100]. The β-CD conjugates with galactose exhibited higher recognition by the galactose-specific *K. bulgaricus* cell wall lectin (KbCWL); i.e., they inhibited flocculation of *K. bulgaricus* cells induced by the isolated KbCWL lectin and their inhibition activity was higher than that of galactose, whereas the glucose derivatives showed no inhibition effect. α-Glucosylgluconoamide–β-CD showed a high affinity (association constant, 8730 M^{-1}) to the glucose-binding protein concanavalin A, a representative of a large family of lectins [101]. It is reported that some galactose and fucose conjugates have a significant cytotoxic effect on the human rectal adenocarcinoma cell line, with P-glycoprotein-positive multidrug resistance [102]. The sugar-substituted CD derivatives may offer a new way of delivering drugs selectively to specific cell surfaces of organs such as liver and colon, although the uptake of drugs into cells may decrease due to the presence of a bulky, hydrophilic CD moiety, as reported for CD conjugates with oligonucleotide and doxorubicin [103,104].

Recently, Péan et al. [105,106] reported that β- and γ-CD derivatives coupled to the neuropeptide substance P bind to the neurokinin 1 receptor (substance P receptor) of rat brain and cultured Chinese hamster ovary (CHO) cells transfected with the human neurokinin 1 receptor gene, in vitro. Furthermore, in vivo intracerebral injection of the substance P–γ-CD conjugate in the rat striatum induced a massive internalization of the receptor, which was monitored by immunofluorescene after labeling of the receptor with a fluorescent polyclonal antibody. Therefore, the immunoreactivity is translocated from plasma membrane to endosomes in dendrites and cell bodies. These results indicate that the substance P moiety bound to γ-CD recognizes the neurokinin receptor-bearing neurons. Schaschke et al. prepared the β-CD conjugates linked covalently to tetra- and hepta-peptide analogues of gastrin through succinyl amide bond and investigated their binding characteristics of G-protein-coupled CCK-B/gastrin receptor expressed in CHO cells [107]. The receptor affinity of the heptapeptide-β-CD conjugate was identical to that of the unconjugated tetrapeptide, although it was weaker than that of the corresponding unconjugated heptapeptide. However, the functional assay monitored by inositol phosphate production was potent as the corresponding parent peptides. They concluded by using docking experiments of the conjugates into the receptor model that the high hormonal potency of the heptapeptide conjugate may be ascribed to a synergetic effect of the specific intermolecular interaction of the peptide with the receptor and unspecific interaction of the β-CD moiety with the receptor surface. These results suggest that rationally designed CD conjugates of bioactive components will provide an attractive means for the targeting of specific cells such as tumor cells.

4. Conclusions

The purpose of this contribution was to outline how well the CDs satisfied the requirements for a drug carrier in drug delivery systems (DDSs). A desirable attribute for the drug carrier is the ability to control the rate and time of drug release; peracylated

CDs may serve as novel hydrophobic carriers to control the release rate of water-soluble drugs. On the other hand, amphiphilic or ionizable CDs can modify the rate or time of drug release, and bind to the surface membrane of cells, which may be used for the enhancement of drug absorption across biological barriers. Moreover, a combination of molecular encapsulation with other pharmaceutical excipients is effective and provides a valuable tool for improving the carrier properties of CDs. The final requirement of the drug carrier is its ability to deliver a drug to a targeted site; CD–drug conjugates may fulfil this requirement for the construction of colon-specific delivery systems. In conclusion, CDs are potential drug carriers in the development of advanced dosage forms; however, most of them are only at the beginning of safety evaluation. The future should see a growth in the number of commercial products using CD-based formulations.

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