Cyclodextrin Drug Carrier Systems

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Contents

I. Introduction

The primary purpose of drug delivery systems is to deliver the necessary amount of drug to the targeted site for a necessary period of time, both efficiently and precisely.[1−3] To design advanced dosage forms, suitable carrier materials are used to overcome the undesirable properties of drug molecules. Hence various kinds of high-performance biomaterials are being constantly developed, with the viewpoint of drug delivery systems in mind.[4] Cyclodextrins are potential candidates for such a role, because of their ability to alter physical, chemical, and biological properties of guest molecules through the formation of inclusion complexes. The α-, *β*-, and *γ*-cyclodextrins are widely used natural cyclodextrins, consisting of six, seven, and eight D-glucopyranose residues, respectively, linked by α-1,4 glycosidic bonds into a macrocycle. Each cyclodextrin has its own ability to form inclusion complexes with specific guests, an ability which depends on a proper fit of the guest molecule into the hydrophobic cyclodextrin cavity.[5−7] The most common pharmaceutical application of cyclodextrins is to enhance the solubility, stability, and bioavailability of drug molecules.[8−11] However, natural cyclodextrins have relatively low solubility, both in water and organic solvents, which thus limits their uses in pharmaceutical formulations. Recently, various kinds of cyclodextrin derivatives have been prepared so as to extend the physicochemical properties and inclusion capacity of natural cyclodextrins as novel drug carriers.[12−19] Thus, the objective of this contribution is to focus on the potential use of chemically modified cyclodextrins as high-performance drug carriers in drug delivery systems with emphasis on the more recent developments. For further information on cyclodextrins and their pharmaceutical application the reader is referred to many excellent books and reviews published in recent years.[20−29] The chemical structures of cyclodextrins and their abbreviations used in this review are shown in Table 1.

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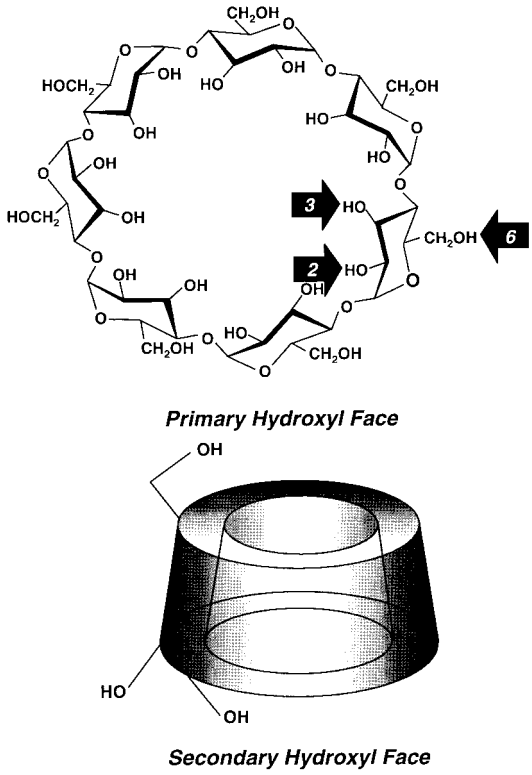
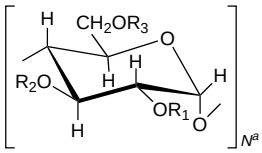


Figure 1. Hydroxyls located on the edge of β-cyclodextrin ring.

II. Characteristics of Cyclodextrins as Drug Carriers

The principal advantages of natural cyclodextrins as drug carriers are the following: (1) well-defined chemical structure, yielding many potential sites for chemical modification or conjugation; (2) availability of cyclodextrins of different cavity size; (3) low toxicity and low pharmacological activity; (4) certain water solubility; (5) protection of included/conjugated drugs from biodegradation. β-Cyclodextrin, the most common natural cyclodextrin, has 21 hydroxyl groups, that is, 7 primary and 14 secondary hydroxyls (Figure 1). These are available as starting points for structural modifications, and various functional groups have been introduced to modify the physicochemical properties and inclusion ability of the parent host molecule. Typical examples of the pharmaceutically useful β-cyclodextrin derivatives are listed in Table 1, classified into hydrophilic, hydrophobic, and ionic derivatives.[20] These cyclodextrin derivatives must be thoroughly characterized before practical use in pharmaceutical dosage forms. In this section, therefore, some physicochemical and biological profiles of cyclodextrin derivatives are described, comparing them with those of natural cyclodextrins.

Table 1. Chemical Structures of Cyclodextrin Derivatives in This Review



compd	R_1	R_2	R_3
Hydrophilic Derivatives			
methylated cyclodextrins			
3-mono-*O*-methylcyclodextrins	H	CH_3	H
2,6-di-*O*-methylcyclodextrins	CH_3	H	CH_3
2,3,6-tri-*O*-methylcyclodextrins	CH_3	CH_3	CH_3
randomly methylated cyclodextrins		R_1, R_2, R_3 = H or CH_3	
hydroxylalkylated cyclodextrins			
2-hydroxyethylcyclodextrins		R_1, R_2, R_3 = H or CH_2CH_2OH	
2-hydroxypropylcyclodextrins		R_1, R_2, R_3 = H or $CH_2CH(OH)CH_3$	
3-hydroxypropylcyclodextrins		R_1, R_2, R_3 = H or $CH_2CH_2CH_2OH$	
2,3-dihydroxypropylcyclodextrins		R_1, R_2, R_3 = H or $CH_2CH(OH)CH_2OH$	
branched cyclodextrins			
6-*O*-glucosylcyclodextrins	H	H	H or glucose
6-*O*-maltosylcyclodextrins	H	H	H or maltose
6-*O*-dimaltosylcyclodextrins	H	H	H or $(maltose)_2$
Hydrophobic Derivatives			
alkylated cyclodextrins			
2,6-di-*O*-ethylcyclodextrins	C_2H_5	H	C_2H_5
2,3,6-tri-*O*-ethylcyclodextrins	C_2H_5	C_2H_5	C_2H_5
acylated cyclodextrins			
2,3-di-*O*-hexanoylcyclodextrins	COC_5H_{11}	COC_5H_{11}	H
2,3,6-tri-*O*-acetylcyclodextrins	$COCH_3$	$COCH_3$	$COCH_3$
2,3,6-tri-*O*-propanoylcyclodextrins	COC_2H_5	COC_2H_5	COC_2H_5
2,3,6-tri-*O*-butanoylcyclodextrins	COC_3H_7	COC_3H_7	COC_3H_7
2,3,6-tri-*O*-valerylcyclodextrins	COC_4H_9	COC_4H_9	COC_4H_9
2,3,6-tri-*O*-hexanoylcyclodextrins	COC_5H_{11}	COC_5H_{11}	COC_5H_{11}
2,3,6-tri-*O*-octanoylcyclodextrins	COC_7H_{15}	COC_7H_{15}	COC_7H_{15}
Ionizable Derivatives			
anionic cyclodextrins			
6-*O*-(carboxymethyl)cyclodextrins	H	H	H or CH_2COONa
6-*O*-(carboxymethyl)-*O*-ethylcyclodextrins	C_2H_5	C_2H_5	H, C_2H_5 or CH_2COONa
cyclodextrin sulfates		R_1, R_2, R_3 = H or SO_3Na	
sulfobutylcyclodextrins		R_1, R_2, R_3 = H or $(CH_2)_4SO_3Na$	

[a] N = 6, α-CDs; N = 7, β-CDs; N = 8, γ-CDs; N = 9, δ-CDs.

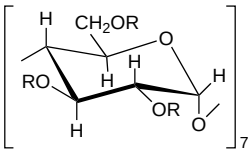
A. Physicochemical Profiles of Cyclodextrins

1. Solubility

Practical use of natural cyclodextrins (α-, β-, and γ-cyclodextrins) as drug carriers is restricted by their low aqueous solubility, particularly that of β-cyclodextrin. The aggregation of cyclodextrins and the interaction of surrounding water molecules, together with the lattice energy in the solid state, may be responsible for the solubility difference of cyclodextrins.[30] Methylation or hydroxyalkylation of the hydroxyl groups of β-cyclodextrin has been used to solve these problems. For example, hydroxyalkylated cyclodextrins are amorphous mixtures of chemically related components with different degrees of substitution.[31−35] This multicomponent character prevents any crystallization, and thus the hydroxyalkylated cyclodextrins have higher solubility (>50%, w/v) in both water and ethanol. The different dissolution mechanism may result in the different temperature dependence of the solubility of hydrophilic cyclodextrins. The solubility of β-cyclodextrin in water shows a normal temperature dependence, i.e., an endothermic dissolution in which solubility increases with increase in temperature. On the other hand, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin shows an exothermic dissolution in water in which the solubility decreases with increase in temperature, because of the dehydration at elevated temperatures.[11,14,15] Thus, methylated cyclodextrins have clouding points, a behavior similar to that of nonionic surfactants. The solubilities of 6-*O*-maltosyl-β-cyclodextrin,[36−38] and 2-hydroxypropyl-β-cyclodextrin,[39] show a slight temperature dependence. Such a property is particularly useful for designing of aqueous injectable cyclodextrin solutions which are to be heat-sterilized.

Controlling the degree of substitution is important in balancing water solubility and complexing capability: increasing the degree of substitution improves aqueous solubility but impairs complexing capacity due to the steric hindrance of the host molecule. For example, the aqueous solubility of 2-*O*-[(*S*)-2-hydroxypropyl]-β-cyclodextrin is lower than that of parent β-cyclodextrin. The crystal structure of the monosubstituted 2-hydroxypropyl-β-cyclodextrin shows that 2-hydroxypropyl group of 1 molecule is inserted into the cavity of an adjacent cyclodextrin

Table 2. Physicochemical Properties of Acylated β-Cyclodextrins



compd	R	melting point (°C)	$[M]_D$[a]	solubility[b] (mg/dL)
β-cyclodextrin	H	280	+1850[d]	119.0
heptakis(2,3,6-tri-*O*-acetyl)-β-cyclodextrin	$COCH_3$	201−202	+2522	823.0
heptakis(2,3,6-tri-*O*-propanoyl)-β-cyclodextrin	COC_2H_5	168−169	+2450	423.5
heptakis(2,3,6-tri-*O*-butanoyl)-β-cyclodextrin	COC_3H_7	126−127	+2607	219.8
heptakis(2,3,6-tri-*O*-valeryl)-β-cyclodextrin	COC_4H_9	54−56	+2640	283.0
heptakis(2,3,6-tri-*O*-hexanoyl)-β-cyclodextrin	COC_5H_{11}	*c*	+2620	3.7
heptakis(2,3,6-tri-*O*-octanoyl)-β-cyclodextrin	COC_7H_{15}	*c*	+2763	*e*
heptakis(2,3,6-tri-*O*-decanoyl)-β-cyclodextrin	COC_9H_{19}	*c*	+2668	*e*
heptakis(2,3,6-tri-*O*-lauroyl)-β-cyclodextrin	$COC_{11}H_{23}$	*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.

leading to a tightly packed crystal lattice and possibly explains the low intrinsic solubility of 2-hydroxypropyl-β-cyclodextrin with low degrees of substitution.[40]

When hydroxyl groups of cyclodextrins are substituted by alkyl groups longer than the methyl group through an ether or an ester linkage, the solubility of cyclodextrins in water decreases proportionally to their degrees of substitution. Among the alkylated cyclodextrins, the ethylated β-cyclodextrins such as heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin and heptakis-(2,3,6-tri-*O*-ethyl)-β-cyclodextrin have been applied as slow-release carriers of water-soluble drugs.[41−43] In a series of peracylated β-cyclodextrins, where all hydroxyl groups of β-cyclodextrin are acylated with different alkyl chains (acetyl−lauroyl), heptakis-(2,3,6-tri-*O*-acetyl)- to heptakis(2,3,6-tri-*O*-valeryl)-β-cyclodextrins were obtained as white crystals, and the melting point decreases in that order (Table 2).[44] On the other hand, the compounds higher than heptakis(2,3,6-tri-*O*-hexanoyl)-β-cyclodextrin occurred as colorless oils. Figure 2 shows solubility curves of various β-cyclodextrin derivatives in the ethanol−water mixture, compared with the parent β-cyclodextrin. With decreasing solvent polarity, the solubility of heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin and heptakis(2,3,6-tri-*O*-acyl)-β-cyclodextrin increased with higher levels of hydrophobicity of the substituent, while that of parent β-cyclodextrin tended to decrease.

In anionic cyclodextrins, the anionic substituents are salts of carbon- and sulfur-based acids. For example, the aqueous solubility of sodium salt of 6-*O*-(carboxymethyl)-β-cyclodextrin is greater than 20 g/dL, but its solubility drops as the pH of the solution decreases.[32] Uekama and co-workers combined the carboxymethyl substituent with an ethylated cyclodextrin to produce 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin, in which an average degree of substitution is 1.8 for the carboxymethyl group and 10.5 for the ethyl substituent. This compound is only slightly soluble in low-pH regions but freely soluble in neutral and alkaline regions due to the ionization of the carboxyl group ($pK_a \approx 3.7$). Thus, 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin can serve as

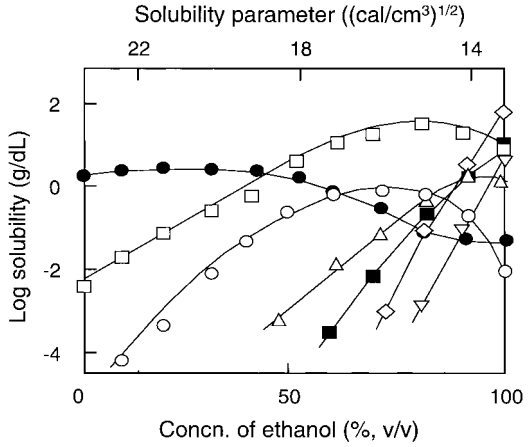


Figure 2. Solubility profiles of β-cyclodextrin derivatives as a function of ethanol concentration in water at 25 °C or solubility parameter of solvent: ●, β-cyclodextrin; □, heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin; ○, heptakis(2,3,6-tri-*O*-acetyl)-β-cyclodextrin; △, heptakis(2,3,6-tri-*O*-propanoyl)-β-cyclodextrin; ■, heptakis(2,3,6-tri-*O*-butanoyl)-β-cyclodextrin; ◇, heptakis(2,3,6-tri-*O*-valeryl)-β-cyclodextrin; ▽, heptakis(2,3,6-tri-*O*-hexanoyl)-β-cyclodextrin.

an enteric-type drug carrier similar to carboxylmethylethylcellulose but may be of greater advantage than the cellulose derivatives for the stabilization of labile drugs owing to the inclusion ability.[46] This derivative was also useful for its ability to slow the release of hydrophilic drugs such as theophilline[47] and for transdermal delivery of prostaglandin E_1.[48,49]

While carboxylated cyclodextrins have properties that vary with pH, the sulfated and sulfonated derivatives are always completely ionized under pH conditions employed in pharmaceutical preparations (pH 3−10). Studies on anionic cyclodextrins suggest that ionic derivatives can be effective complexing agents if the charge is spaced away from the cyclodextrin cavity by neutral spacer groups.[28] The best host molecule for the sulfonate derivatives seems to be a sulfobutyl-β-cyclodextrin, because this compound effectively bind drugs with minimal disturbances caused by varying the degree of substitution. In particular, sulfobutyl-β-cyclodextrin, with an average molar degree of substitution of ∼7 for the sulfobutyl

ether group, has a high intrinsic aqueous solubility (>50%, w/v) and exhibits binding capacities comparable to its parent, β-cyclodextrin.[28]

2. Stability

The glycosidic bonds of cyclodextrins are fairly stable in alkaline solution, whereas they are hydrolytically cleaved by strong acids to give linear oligosaccharides.[5,7] Furthermore, they are more resistant to acid-catalyzed hydrolysis, compared with that of linear sugars, and the ring-opening rate of cyclodextrins increases with increasing cavity size (α-cyclodextrin < β-cyclodextrin < γ-cyclodextrin). This reactivity difference is clearly seen in the hydrolysis of 6-*O*-maltosyl-β-cyclodextrin, which has three types of glycosidic bonds, i.e., α-1,4 glycosidic bond in the cyclodextrin ring, α-1,4 bond in the linear maltosyl residue, and α-1,6 glycosidic bond at a junction between the ring and the branched sugar. Thus, the hydrolysis rate of one glycosidic bond in 6-*O*-maltosyl-β-cyclodextrin decreases in the order of α-1,4 bond in the linear sugar > α-1,4 bond in the ring ≫ α-1,6 bond at the junction.[50] The ring-opening rate of cyclodextrins is accelerated when the ring is distorted; i.e., heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin having a distorted ring conformation is more susceptible to acid-catalyzed hydrolysis than the parent β-cyclodextrin and heptakis(2,6-di-*O*-methyl)-β-cyclodextrin.[15] It should be noted that the ring-opening rate of β-cyclodextrin is decelerated by the addition of guest molecules, the deceleration being marked for guests with a close fit of the β-cyclodextrin cavity.[51] This deceleration can be attributed to the inhibition of access of catalytic oxonium ions to the glycosidic bond, because the cyclodextrin cavity is occupied by guests. The glycosidic bonds of cyclodextrins are cleaved by some starch-degrading enzymes with the proper substrate specificity, although its reaction rate is much slower than that of linear sugars.[38] Thus, parent cyclodextrins are hydrolyzed by α-amylase at an appreciable rate (α-cyclodextrin < β-cyclodextrin < γ-cyclodextrin), whereas they are not hydrolyzed by glucoamylase that cleaves the α-1,4 glycosidic bond from terminal nonreducing glucose and pullulanase that cleaves the α-1,6 glycosidic bond. On the other hand, 6-*O*-maltosyl-β-cyclodextrin is a good substrate for glucoamylase and pullulanase and is hydrolyzed at a very slow rate by α-amylase because of the steric hindrance of branched sugar moieties. Generally, the introduction of substituents on the hydroxyl groups slows down enzymatic hydrolyses of cyclodextrins by lowering the affinity of cyclodextrins to enzymes or reducing the intrinsic reactivity of enzymes.

α- and β-cyclodextrins are resistant to the metabolism in the body, whereas γ-cyclodextrin having a large cavity is hydrolyzed even by human salivary α-amylase.[52] On the other hand, β-cyclodextrin is hardly hydrolyzed at all in the whole blood of rats, rabbits, dogs, and humans and also in rat liver homogenates.[53] In sharp contrast, 6-*O*-maltosyl-β-cyclodextrin having a α-1,4 glycosidic bond in the branched sugar is easily hydrolyzed to give 6-*O*-glucosyl-β-cyclodextrin in the blood of rats and dogs, while it is resistant in that of rabbits and humans. This interspecies difference in the metabolism of 6-*O*-maltosyl-β-cyclodextrin is ascribable to the different activity of glucose-forming amylase including glucoamylase in various mammals. Thus, the α-1,4 glycosidic bonds in the cyclodextrin ring and the α-1,6 bond at a junction between the cyclodextrin ring and the substituents are hydrolytically stable in human body fluids, and the branched β-cyclodextrins will be excreted as intact forms in urine.

B. Biological Profiles of Cyclodextrins

1. Bioadaptability

To realize the potential of cyclodextrins in pharmaceutical formulations, their relevant biological profiles including in-vivo fate and toxic effects have to be clarified. The metabolic fate of natural cyclodextrins given orally has been thoroughly investigated, and their lack of toxicity has been well documented.[54] However, few attempts have been made at a comprehensive evaluation of the majority of chemically modified cyclodextrins. For example, subacute or subchronic intravenous administration of 2-hydroxypropyl-β-cyclodextrin to rats and monkeys showed no significant alteration in the morphological and clinical pathology parameters.[55] When hydrophilic cyclodextrins were administered intravenously to rats, they disappeared rapidly from the plasma.[53,56] β-Cyclodextrin and 2-hydroxypropyl-β-cyclodextrin were recovered almost completely in the intact form (>95%), indicating that there is no significant metabolism. On the other hand, the rapid disappearance of 6-*O*-maltosyl-β-cyclodextrin from the blood in rats was accompanied by the enzymatic conversion into 6-*O*-glucosyl-β-cyclodextrin in the urine. However, 6-*O*-maltosyl-β-cyclodextrin was excreted as soluble 6-*O*-glucosyl-β-cyclodextrin, not as less soluble β-cyclodextrin. The nephrotoxicity of natural β-cyclodextrin at higher doses was ascribed to the crystallization of less soluble β-cyclodextrin or its cholesterol complex in renal tissue.[57] Therefore, the metabolic fates of 6-*O*-maltosyl-β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin are suggestive of less renal toxicity, compared with those of parent β-cyclodextrin. To assess the tolerance via the parenteral administration route, various blood chemistry parameters in rats and rabbits after the multiple intravenous administrations of hydrophilic β-cyclodextrins were compared with those of parent β-cyclodextrin. The multiple injections of β-cyclodextrin or heptakis(2,6-di-*O*-methyl)-β-cyclodextrin at a total dose of 900 mg/kg in rats and 1200 mg/kg in rabbits increased the blood urea nitrogen, creatinine, glutamate oxaloacetate transaminase, and glutamate pyruvate transaminase, indicating some kidney and liver failure, while those for 2-hydroxypropyl-β-cyclodextrin and 6-*O*-maltosyl-β-cyclodextrin at the same dose remained within normal limits. Moreover, no noticeable changes were detected in the volume of urine and the amounts of proteins excreted in urine for 24 h after the multiple intravenous administrations of these β-cyclodextrins in rats. Furthermore, recent studies have demonstrated the lack of

toxicity of cyclodextrin sulfates when given intravenously and intramuscularly to rats.[20] These facts suggest that 2-hydroxypropyl-β-cyclodextrin, 6-*O*-maltosyl-β-cyclodextrin, and β-cyclodextrin sulfate can be safely used in parenteral formulations.

Cyclodextrins are known to induce human erythrocytes to change their biconcave shape to monoconcave and at higher concentrations induce the lysis. The hemolytic activity of natural cyclodextrins is reported to be in the order of β- > α- > γ-cyclodextrin.[58,59] These differences were ascribed to the differential solubilization of membrane components by each cyclodextrin. The process of solubilization occurs without entry of cyclodextrins into the membranes, a mechanism of solubilization/lysis different from that of detergents, which enter the membranes. A similar solubilization process is reported for the cyclodextrin-induced lysis of the artificial membranes composed of lecithin and cholesterol.[60] When the cyclodextrin cavity is modified by chemical derivatization, its effects on cell membranes can be dramatically changed in a manner different from that of parent cyclodextrins.[33,38] The muscle tissue damage due to the injection of hydrophilic cyclodextrins was compared with that of mannitol and nonionic surfactants, following a single injection (100 mg/mL) of the compounds into *M. vastus lateralis* of rabbits. α-Cyclodextrin and heptakis(2,6-di-*O*-methyl)-β-cyclodextrin showed a relatively high irritation reaction, the degree of which corresponded to that of a detergent Tween 80. On the other hand, 6-*O*-maltosyl-β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, and cyclodextrin sulfates showed no or only slight irritation reactions, the degree of which was comparable to those of γ-cyclodextrin, mannitol, and a detergent HCO-60. The local irritation of the highly hydrophilic β-cyclodextrins was in parallel with their hemolytic action,[38] suggesting a similar mechanism of the local action, i.e., membrane perturbation.[59]

More promising cyclodextrins for parenteral use will be 2-hydroxypropyl-β-cyclodextrin and sulfobutyl-β-cyclodextrin with an average degree of substitution of ∼7.[28] 2-Hydroxypropyl-β-cyclodextrin has generally been found to be safe when administered parenterally in animals and humans. No adverse effects were observed in the human studies. Sulfobutyl-β-cyclodextrin with an average degree of substitution of ∼7 has also been found to be safe when administered parenterally with doses greater than 10 g/kg causing no toxic effects in mice.[61]

2. Absorption Behavior

In general, cyclodextrins are poorly absorbed from the gastrointestinal tracts following oral administration, because of their bulky and hydrophilic nature. For example, the oral bioavailability of β-cyclodextrin in rats has been reported to range from 0.1%[62] to 4%.[63] The mode of absorption of the cyclodextrins is probably passive[64] with the majority of an orally administered cyclodextrin dose being metabolized by the gastrointestinal flora.[65,66] The in vitro studies using everted rat intestinal sac have demonstrated that intact β-cyclodextrin passed through the intestinal wall by passive diffusion only in slight amounts.[67] Furthermore, recent studies in which the in-situ loop and single perfusion were used revealed that when the complex of a drug with β-cyclodextrin was introduced into the lumen of rat intestine, it can be detected in the circulating blood to a certain extent.[68,69] The effect of bile on the intestinal absorption of natural cyclodextrins in rats was examined using the in-situ loop recirculating perfusion technique.[64] Only a very little of β- and γ-cyclodextrins was absorbed by the intestinal segment under the bile duct ligated condition. However, when sodium taurocholate, one of the major components of rat bile, was present, α-cyclodextrin entered the systemic circulation. Therefore, it seems likely that the trace amounts of cyclodextrins in an intact form can be absorbed from the gastrointestinal tracts, depending on the physiological conditions.

In the case of the rectal route, the situation is much more obvious, compared to gastrointestinal absorption. When the oleaginous suppositories containing β-cyclodextrins (β-cyclodextrin, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, or 2-hydroxypropyl-β-cyclodextrin) were administered to the rat rectum, considerable amounts of intact 2-hydroxypropyl-β-cyclodextrin or heptakis(2,6-di-*O*-methyl)-β-cyclodextrin were excreted into the urine up to 24 h after administration.[70] Moreover, when β-cyclodextrins were coadministered with a drug in vivo, rather high amounts of 2-hydroxypropyl-β-cyclodextrin (>26% of dose) and heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (>21% of dose), compared with β-cyclodextrin (>5% of dose), were absorbed from the rat rectum. The relatively high absorption observed for β-cyclodextrin derivatives was ascribed to the change in the permeability of the rectal mucosa and/or the interaction between the surface active β-cyclodextrins and glycerides, which are principal components of the suppository bases. These glycerides may facilitate the rectal absorption of β-cyclodextrins in the forms of inclusion complexes. Similarly, some ointment bases assist the transdermal absorption of β-cyclodextrins, in particular chemically modified β-cyclodextrins, under the occlusive-dressing conditions, which will be discussed in section III.C.5.

III. Improvements of Drug Properties by Cyclodextrin Complexation

One of the important characteristics of cyclodextrins is the formation of inclusion complexes in both the solution and solid states, in which each guest molecule is surrounded by the hydrophobic environment of the cyclodextrin cavity. This can lead to the alteration of physical, chemical, and biological properties of guest molecules and can eventually have considerable pharmaceutical potential. This section, therefore, deals with recent aspects of the practical use of cyclodextrins in various pharmaceutical formulations and will discuss some fundamental characteristics of cyclodextrins which should be considered in the development of advanced dosage forms.

A. Solubilization

One of the most important applications of cyclodextrins in pharmaceutical fields is to enhance aque-

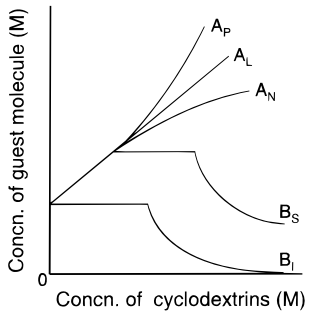


Figure 3. Type of phase solubility diagram.

ous solubility of drugs through inclusion complexation. The solubilization ability of cyclodextrins can be quantitatively evaluated by the phase solubility method developed by Higuchi and Connors.[71] The phase solubility diagrams, i.e., plots of solubility of guest as a function of cyclodextrin concentration, are generally classified as either type A (a soluble complex is formed) or type B (a complex with definite solubility is formed), as shown in Figure 3. The type A can be further classified in subtypes A_L, A_P, and A_N, where the guest solubility of the first type increases linearly with cyclodextrin concentration while those of the second and third types deviate positively and negatively, respectively, from the straight line. The complex formation with a 1:1 stoichiometry gives the A_L type diagram, whereas the higher order complex formation in which more than one cyclodextrin molecules are involved in the complexation gives the A_P type. The interaction mechanism for the A_N-type is complicated, because of a significant contribution of solute−solvent interaction to the complexation. In the case of the B_S type, the initial ascending portion of the solubility change is followed by a plateau region and then a decrease in the solubility at higher cyclodextrin concentrations, accompanying a microcrystalline precipitation of the complex. The B_I-type diagram is indicative of the formation of insoluble complexes in water. The stability constant and stoichiometry of complexes are determined by analyzing quantitatively the phase solubility diagram. The solid cyclodextrin complexes can be prepared by referring the B-type solubility diagram. This section is concerned with the solubilization of poorly water-soluble drugs by the recently developed cyclodextrin derivatives with high aqueous solubility, i.e., hydroxyalkylated, sulfated, sulfoalkylated, and branched cyclodextrins and by a combination of cyclodextrin and additives.

1. Hydroxyalkylated Cyclodextrins

Müller and Brauns compared the solubilizing effect of 2-hydroxyethyl-β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin with different degrees of substitution on poorly water-soluble drugs such as hydrocortisone, digitoxin, diazepam, and indomethacin.[32] The solubilization by parent β-cyclodextrin is limited because these guests conformed to show the B_S type phase solubility diagram in which the complexes crystallize at higher cyclodextrin concentrations (1∼2%, w/v) and because β-cyclodextrin has limited solubility (∼2%, w/v). On the other hand, these hydroxyalkylated β-cyclodextrins demonstrated the A_L-type diagram pattern wherein the guest solubility increases linearly up to ∼10% (w/v) concentration of the hosts (the intrinsic solubility of the hosts, >50%, w/v). The solubilizing effect of the 2-hydroxyethyl-β-cyclodextrin with an average degree of substitution of 3.0 was almost comparable to that of 2-hydroxypropyl-β-cyclodextrin with an average degree of substitution of 2.5: the solubilities of digitoxin in the presence of 1.8% (w/v) parent β-cyclodextrin, 5% (w/v) 2-hydroxyethyl-β-cyclodextrin, and 5% (w/v) 2-hydroxyethyl-β-cyclodextrin were 0.8, 9.5, and 8.6 mg/mL, respectively. On the other hand, the degree of substitution markedly influences the solubilization: the solubility of digitoxin in the presence of 5% (w/v) 2-hydroxyethyl-β-cyclodextrin with an average degree of substitution of 11.0 decreased to 1.7 mg/mL. Our results on 3-hydroxypropyl-β-cyclodextrin with an average degree of substitution of 6.1 and 2,3-dihydroxypropyl-β-cyclodextrin with an average degree of substitution of 5.9 showed that the latter derivative has a lower solubilizing ability than parent β-cyclodextrin, whereas the former derivative has a slightly higher ability.[34] The stability constants of the complexes with digitoxin were 20 000 M^{-1} (3-hydroxypropyl-β-cyclodextrin) > 17 000 M^{-1} (parent β-cyclodextrin) > 14 000 M^{-1} (2,3-dihydroxypropyl-β-cyclodextrin). The decrease in the complexing ability of 2,3-dihydroxypropyl-β-cyclodextrin may be due to a steric hindrance of the dihydroxypropyl group, whereby this above-mentioned negative effect is compensated by the enhanced complexing ability of 3-hydroxypropyl-β-cyclodextrin due to increase in hydrophobicity of the substituent. Such significant effects of the degree of substitution on the solubilizing ability of 2-hydroxypropyl-β-cyclodextrin, which is a most popular and widely used hydroxyalkylated derivative, are reported by Pitha and co-workers[72] and Loftsson and co-workers.[73] Two pharmaceutical products containing 2-hydroxypropyl-β-cyclodextrin are on market; one is an aqueous mouthwash solution containing 0.3% (w/v) hydrocortisone solubilized by 4% (w/v) 2-hydroxypropyl-β-cyclodextrin (trade name: DEXACORT, Reykjavik, Iceland), which is used for treatment of recurrent ulceration and erosive lichen planus of oral mucosa.[74] Another one is the itoraconazole liquid preparations (trade name: SPORANOX, Beerse, Belgium) which is used for treatment of esophageal candidiasis.[75]

2. Sulfated and Sulfoalkylated Cyclodextrins

Cyclodextrin sulfates are cyclodextrin derivatives in which a majority of their hydroxyl groups are sulfated and have been shown to possess potentially important biological activities similar and sometimes superior to those of heparin.[76] Because both cyclodextrin rims are surrounded by negatively charged groups, cyclodextrin sulfates interact with positively charged drug molecules such as chlorpromazine[77] and gentamicin.[78] Recent interaction studies[77] of cyclodextrin sulfates indicated that both hydrophobic and electrostatic interactions take part in the complexation. Table 3 shows the stability constants of chlorpromazine with parent β-cyclodextrin and β-cyclodextrin sulfate with an average degree of substitution of 12.0 and sulfobutyl-β-cyclodextrin sulfate with

Table 3. Stability Constants (K_c) of Complexes of Chorpromazine with β-Cyclodextrins and Thermodynamic Parameters in Isotonic Phosphate Buffer (pH 7.4) at 25 °C

system	K_c (M^{-1})	ΔG ($kJ \cdot mol^{-1}$)	ΔH ($kJ \cdot mol^{-1}$)	ΔS ($J \cdot K^{-1} \cdot mol^{-1}$)
β-cyclodextrin	9050	−22.6	−28.3	−19.3
β-cyclodextrin sulfate[a]	1640	−18.3	−12.7	18.8
sulfobutyl-β-cyclodextrin[b]	16100	−24.0	−24.0	0.07

[a] The average degree of substitution of 12.0. [b] The average degree of substitution of 3.9.

an average degree of substitution of 3.9 at 25 °C and their thermodynamic parameters. The inclusion ability of β-cyclodextrin sulfate with chlorpromazine was weaker than that of parent β-cyclodextrin. The larger positive ΔS change (18.8 J K^{-1} M^{-1}) of β-cyclodextrin sulfate suggests that some water molecules are released from the ionic binding sites by the complexations. Unfortunately, the solubilization ability of cyclodextrin sulfates has not been subjected to detailed investigation because of the relatively weak interactions with acidic and neutral drugs such as flufenamic acid, flurbiprofen, diazepam, and steroids.[77] The weak interaction may due to the difficulty in including hydrophobic guests into the cavity through the highly hydrated entrance as well as the electrostatic repulsion between negative changes of the host and anionic guest. In contrast, less soluble complexes of cyclodextrin sulfates with cationic drugs are sometimes formed, because of the dehydration of the complexes resulting from the charge neutralization.[78]

To overcome such a drawback with the cyclodextrin sulfates, a series of sulfoalkyl ether derivatives have been prepared, in which the sulfonate groups are appropriately spaced from the cyclodextrin cavity with alkyl groups.[79] The inclusion ability of sulfoalkylated cyclodextrins is dependent not only on the degree of substitution of the substituent but also on the spacer length. Stella and co-workers investigated the inclusion ability of sulfopropyl-β-cyclodextrin and sulfobutyl-β-cyclodextrin with different degrees of substitution against testosterone and progesterone and reported the following stability constants (M^{-1}): for testosterone 16 100 (SPE1), 33 400 (SPE4), 19 400 (SPE6), 18 100 (SBE1), 38 100 (SBE4), 42 100 (SBE7), and 41 700 (SBE12) and for progesterone 20 700 (SPE1), 23 700 (SPE4), 14 400 (SPE6), 24 500 (SBE1), 30 400 (SBE4), 34 000 (SBE7), and 29 500 (SBE12), where SPE and SBE represents sulfopropyl-β-cyclodextrin and sulfobutyl-β-cyclodextrin, respectively, and the number in parentheses is the average degree of substitution.[80] The stability constants of testosterone with parent β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin are 17 800 and 14 700 M^{-1} under the same conditions. It is concluded from these results that the binding potential increases with increasing the spacer chain length because the charged groups of sulfobutyl-β-cyclodextrin are appropriately spaced from the cavity and the hydrophobicity around the cavity increases due to the presence of the alkyl chain. Furthermore, there is an optimum number of sulfoalkyl groups on the cyclodextrin torus; thus, the larger degree of substitution results in inhibition of complexation. As shown in Table 3, chlorpromazine forms the inclusion complex with sulfobutyl-β-cyclodextrin more strongly than with parent β-cyclodextrin.[81] It is reported that sulfobutyl-β-cyclodextrin is a good solubilizer for various poorly water-soluble drugs such as kynostatin, steroids, pilocarpine, cinnarizine, indomethacin, naproxen, warfarin, papaverin, thiabendazole, miconazole, etc.[28]

3. Branched Cyclodextrins

When mono- or disaccharides are introduced onto one or two primary hydroxyl groups of cyclodextrins through the α-1,6 glycosidic bond, their solubility in water markedly increases: the solubility of 6-*O*-glucosyl-β-cyclodextrin, 6-*O*-maltosyl-β-cyclodextrin and 6^A,6^D-di-*O*-α-maltosyl-β-cyclodextrin in water at 25 °C is over 50% (w/v).[38] These branched cyclodextrins are enzymatically prepared and obtained as a single component, in contrast to hydroxyalkylated and sulfoalkylated derivatives.[82] The X-ray structure determination of 6-*O*-glucosyl-α-cyclodextrin indicated that the glucose unit incorporated onto the primary hydroxyl group extends outside the cavity and parallel to the α-cyclodextrin wall and is similar to the lid of an opened can thus keeping the cavity open.[83] The inclusion ability of branched cyclodextrins against hydrophobic guest molecules is comparable to that of parent cyclodextrins and decreases only slightly with increase in the glucose number and the degree of substitution.[37,38,84] However, the solubilization effect of branched cyclodextrins is much greater than that of parent cyclodextrins because solutions concentrated over 50% can be set up. The phase solubility diagram of branched cyclodextrins with various drug molecules generally conforms with either the A_L or A_P type, indicating soluble complex formation. Branched β-cyclodextrins have higher affinity to drugs having steroid skeletons such as progesterone, testosterone, dehydrocholic acid, digitoxigenin, digitoxin, etc.[38,85] However, some long-chain fatty acids[84] and fucosterol[86] show the B_S-type diagram with 6-*O*-glucosyl-α-cyclodextrin and 6-*O*-maltosyl-β-cyclodextrin, respectively, although precipitation of the solid complexes occurred only at much higher concentrations of the guests. Therefore, branched cyclodextrins may be useful as solubilizers for parenteral preparations such as injections, because of their high solubilizing ability, weak hemolytic activity, and high bioadaptability.[53]

4. Combination of Cyclodextrins with Additives

As described before, the utility of β-cyclodextrin is limited by its low intrinsic solubility coupled with a decrease in solubility on complexation. One approach taken to overcome such aqueous solubility problems is to prepare water-soluble derivatives. On the other hand, the solubility of cyclodextrins is significantly affected by water-miscible cosolutes and cosolvents.

Urea is known to increase water solubility of a variety of nonpolar and polar organic solutes, probably by modifying water structure. This compound increases significantly the β- and γ-cyclodextrin solubilities: the enhancement in the presence of 7 M urea is about 11 and 2.3 for β- and γ-cyclodextrins, respectively.[87] On the other hand, the solubility of α-cyclodextrin is decreased by the addition of urea. Such solubility changes are observed for cosolvent systems such as alcohols, acetonitrile, dimethyl sulfoxide, dimethyl formamide, ethylene glycol, dimethyl ether, etc.[88,89] Therefore, the cosolute or cosolvent system may affect the inclusion process or equilibrium. Unfortunately, research along this line has not been extensively undertaken. Pitha and co-workers reported that there is no synergetic effect of ethanol on the solubilization of testosterone by 2-hydroxypropylated cyclodextrins, and the stability constant of the complex of testosterone with 2-hydroxypropyl-β-cyclodextrin decreases linearly from about 104 M^{-1} in water to below 1 M^{-1} in 80% (v/v) ethanol/water solution.[90] However, the cosolubilization method is useful for the preparation of solid 2-hydroxypropylated cyclodextrin complexes with unstable drugs such as steroids, peptides, and antibiotics by means of the evaporation and freeze-drying methods. Loftsson and co-workers studied the synergetic enhancing effect of cyclodextrin derivatives (methylated, hydroxyalkylated, carboxymethylated, and branched forms) and water-soluble polymers (polyvinylpyrroridone, hydroxypropylmethylcellulose, and carboxymethylcellulose) on solubility of various poorly water-soluble drugs.[91,92] For example, by the addition of 0.25% (w/v) poly(vinylpyrroridone), the stability constant of the complex of hydrocortisone with 2-hydroxypropyl-β-cyclodextrin increased from 890 to 1070 M^{-1} and that of the 6-*O*-(carboxymethyl)-β-cyclodextrin complex from 3150 to 9000 M^{-1}. Hydroxypropylmethylcellulose (0.1%, w/v) increased the stability constant of the complex of methazolamide with 2-hydroxypropyl-β-cyclodextrin from 28 to 69 M^{-1}. The larger stability constants observed in the ternary systems were reflected in more negative ΔH changes, a part of which was compensated by negative changes of ΔS, suggesting that the effect of polymers on the cyclodextrin complexation is not a simple hydrophobic effect. In general, the solubilizing effect of 2-hydroxypropyl-β-cyclodextrin is enhanced on average by 27% by carboxymethylcellulose and 49% by poly(vinylpyrroridone). The dissolution characteristics of the complex of diazepam with γ-cyclodextrin are improved by the addition of water-soluble polymers.[93] Although the enhancing mechanism of polymers is not fully elucidated, these cosolute and cosolvent systems are of potential as an alternative method for the solubilization of drugs in pharmaceutical formulations.

B. Stabilization

Cyclodextrins are known to accelerate or decelerate various kinds of reactions, exhibiting many kinetic features shown by enzyme reactions, i.e., catalyst−substrate complex formation, competitive inhibition, saturation, and stereospecific catalysis.[5] When an ester group of guest molecules is fixed in close proximity to the catalytic site of cyclodextrins, i.e., secondary hydroxyl groups, it experiences an acceleration in hydrolysis. On the other hand, the hydrolysis is decelerated when the ester group is included deeply inside the cavity. The rate of reactions such as decarboxylations[94] and trans−cis isomerizations[95] is changed by the inclusion because the guest is transferred from a polar environment of water to a less polar one of cyclodextrin cavity, i.e., microsolvent effect. The reaction rate increases when flexible guest molecules are forced to fix in a reactive conformation and vice versa.[96] The drugs must retain sufficient stability not only during storage but also in the gastrointestinal fluids, since reactions which result in a product that is pharmacologically inactive or less active will reduce the therapeutic effectiveness. Therefore, the main concern in pharmaceutical field is the rate deceleration. The stabilization effect of cyclodextrins in solution and the solid state is described in the next subsections.

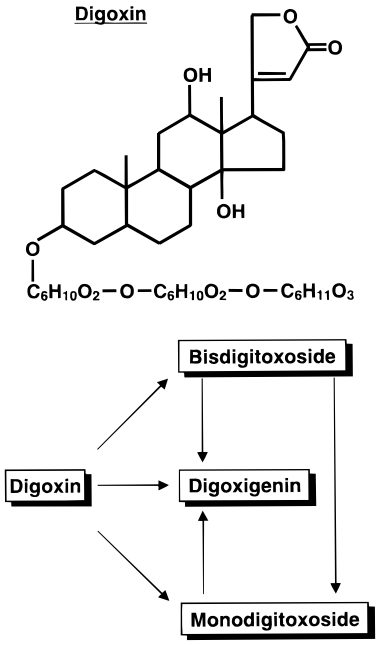


Figure 4. Acid-catalyzed hydrolysis of the glycoside bonds in digoxin.

1. In Solution

Digoxin, one of the potent cardiac glycosides, is susceptible to hydrolysis in acidic media. In this degradation pathway, prevention of the appearance of digoxigenin might be clinically important because the cardioactivity of digoxigenin is about one-tenth of that of digoxin, but other digoxosides (mono- and bis(digoxosides)) possess approximately the same activity (Figure 4). The acid-catalyzed hydrolysis of the glycoside bonds in digoxin are decelerated by the addition of cyclodextrins.[97] In particular, the hydrolysis of the glycosidic linkage connecting the A-ring of digoxin and the sugar is completely inhibited by β-cyclodextrin: the hydrolysis rate constants in the presence of α-, β-, and γ-cyclodextrins (1.0×10^{-2} M) at pH 1.66, 37 °C, are 0.108, 0.002, and 0.025 h^{-1}, respectively, whereas that in the absence of

cyclodextrins is 0.17 h^{-1} (guest concentration 1.0×10^{-4} M). Spectroscopic investigations reveal that the A-ring of digoxin is located at the entrance to the α-cyclodextrin cavity (stability constant 180 M^{-1}), such that it could penetrate further into the β-cyclodextrin cavity (11 200 M^{-1}) and that it is loosely bound to γ-cyclodextrin (12 200 M^{-1}). These indicate that either a small or a large cavity is unfavorable for preventing the hydrolysis of digoxin. The acid-catalyzed degradation of drugs such as prostacyclin is inhibited by cyclodextrins, and the deceleration effect increased with increase in the stability constants.[98] When the catalytic hydroxyl groups of cyclodextrins are blocked by substituents, their stabilizing ability enhances. A typical example is the effects of methylated β-cyclodextrins on degradation of E-type prostaglandins.[99] The β-hydroxyketo moiety of E-type prostaglandins is extremely susceptible to dehydration under acidic or alkaline conditions to give A-type prostaglandins, which are isomerized subsequently to B-type prostaglandins under alkaline conditions. The biological activities of E-type prostaglandins decrease as these reactions progress. Parent cyclodextrins showed a positive catalytic effect, i.e., acceleration, on these degradations. In sharp contrast, methylated β-cyclodextrins showed a significant negative effect which was larger for heptakis(2,6-di-*O*-methyl)-β-cyclodextrin than for heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin, because the former includes more tightly the reactive moiety of E-type prostaglandins than does the latter. When acidic groups such as carboxylic acid are introduced onto the hydroxyl groups of cyclodextrins, the included guest undergoes a profound change in environmental acidity which affects the reactivity. For example, 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin has been used to stabilize prostaglandin E_1 in a fatty alcohol propylene glycol ointment, because prostaglandin E_1 is most stable in weak acidic conditions.[100] Carmofur (1-(hexylcarbamoyl)-5-fluorouracil) is one of the masked forms of 5-fluorouracil and extremely susceptible to base- and water-catalyzed hydrolysis to 5-fluorouracil (the most stable pH is ∼4). The acidic property of 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin is effective in preventing the degradation of carmofur into 5-fluorouracil, which irritates the gastrointestinal tracts, and in improving the oral bioavailability of carmofur.[46] An antitumor drug, O^6-benzylguanine, undergoes acid-catalyzed hydrolysis to form guanine and benzyl alcohol according to an S_N1 mechanism with significant charge separation in the rate-determining step. This hydrolysis at pH 4.8 was decelerated by a factor of 220 by the complexation with sulfobutyl-β-cyclodextrin with an average degree of substitution of ∼4, because of the unfavorable charge separation in the hydrophobic cavity of the cyclodextrin.[101]

Our recent results suggest that the stoichiometry of cyclodextrin complexes affects chemical reactivities of guests. An antiulcer agent, 2′-(carboxymethoxy)-4,4′-bis((3-methyl-2-butenyl)oxy)chalcone, formed the 1:1 complex with cyclodextrins at lower concentrations of the host and the 1:2 (guest:host) complex at higher concentrations.[102] The trans−cis photoisomerization of the antiulcer agent in the 1:1 complex was decelerated whereas that in the 1:2 complex was accelerated. The deceleration of α-cyclodextrin was much greater because the inclusion in the smaller cyclodextrin cavity hinders severely the rotation of the double bond of the cinnamoyl group. On the other hand, the 1:2 γ-cyclodextrin complex most significantly accelerated the reaction. The larger acceleration is due to the complete inclusion of the guest within a γ-cyclodextrin dimer which is less hydrated, because the isomerization of the antiulcer agent is favorable in less polar solvents. An antiallergic agent, tranilast (*N*-(3,4-dimethoxycinnamoyl)anthranilic acid), forms inclusion complexes with γ-cyclodextrin with a different stoichiometry.[103] At relatively low concentrations of γ-cyclodextrin or high concentrations of tranilast, two guest molecules are included in one γ-cyclodextrin cavity, which accelerates the photodimerization of the guest. With increasing γ-cyclodextrin concentration, the 1:1 and 1:2 complexes are formed and the dimerization rate decreases; in particular the 1:2 complex decelerates it by 19 300 times. Therefore, cyclodextrin concentrations should be optimized to allow for a maximal stabilization of guests.

2. In the Solid State

The improvement of chemical stability of drugs in the solid state by cyclodextrin complexation has not been extensively investigated,[9,13,15] because solid-state kinetics are complicated compared to solution kinetics and cyclodextrins sometimes accelerate the water-sensitive degradation rates owing to their high water-sorption property. For instance, the degradation of carmofur in the solid state is accelerated by the complexation with parent β-cyclodextrin, whereas it is inhibited by methylated cyclodextrins which are less water-sorptive.[104] Many solid compounds exist in different crystalline modifications such as amorphous, crystalline, or solvated forms, affecting solubility, dissolution rate, stability, and bioavailability. Among these solid states, amorphous forms are of pharmaceutical interest because they give a significant increase in dissolution and bioavailability of drugs. However, amorphous forms easily transform to a stable crystalline form during handling and storage of drugs. Therefore, it is important to control the crystallization, polymorphic transition, and whisker generation of solid drugs. In this section, we will focus our attention on the effect of cyclodextrins on the physical stability of drugs in the solid state, such as crystallization and polymorphic transition.

Nifedipine, a potent calcium-channel antagonist, is a crystalline drug having low solubility and slow dissolution rate. Solid dispersion of the drug with various hydrophilic macromolecules such as poly(vinylpyrroridone) and poly(ethylene glycol)s has been used to solve such solubility problems.[105] However, amorphous nifedipine gradually crystallizes in these matrixes, during storage at high temperatures and high humidity. Crystalline nifedipine is converted to an amorphous state by spray-drying with amorphous 2-hydroxypropyl-β-cyclodextrin, and the oral bioavailability is improved significantly.[106,107]

2-Hydroxypropyl-β-cyclodextrin is useful in preventing the crystal growth of amorphous nifedipine, maintaining a relatively fine and uniform size of crystals even under adverse storage conditions (60 °C, 75% relative humidity). The size of nifedipine crystals grown in polyvinylpyrroridone matrix was >50 μm, whereas that in 2-hydroxypropyl-β-cyclodextrin matrix after dissociation of the inclusion complex is ~5 μm. Furthermore, 2-hydroxypropyl-β-cyclodextrin can prevent the polymorphic transition of a metastable nifedipine to a stable form. The metastable form (form B, mp 163 °C) of nifedipine is transiently formed at an early stage of storage of its amorphous form, and the glassy form of nifedipine is obtained by cooling melts of the stable form (form A, mp 171 °C). 2-Hydroxypropyl-β-cyclodextrin suppressed the crystallization of the glassy nifedipine and the polymeric transition of form B to form A, indicating that form B could be prepared in high yield (75%) by heating in the amorphous 2-hydroxypropyl-β-cyclodextrin matrix. Such inhibitions of crystallization and polymorphic transition are reported for chloramphenicol palmitate,[108] spironolactone,[109] metronidazole benzoate,[110] and tolbutamide.[111] The 2-hydroxypropylated cyclodextrin complexes with spironolactone maintained an amorphous state for lengthy time periods (over 2 months at 75% relative humidity and 60 °C). The whisker growth from tablets and powder of isosorbide 5-mononitrate, an organic nitrate vasodilator, is retarded by the complexation with β-cyclodextrin.[112] Therefore, the cyclodextrin complexation is also useful for the stabilization of drugs in the solid state.

C. Absorption Enhancement

The rate and extent of bioavailability of a poorly water-soluble drug from its cyclodextrin complex can be optimized by adjusting various factors affecting the dissociation equilibrium of the complex both in the formulation and in the biophase in which the complex is administered. Only a free form of the drug, which is in equilibrium with the complexed one 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, maximal absorption enhancement is obtained when just enough cyclodextrin is used to solubilize all the drug in solution. Further addition of cyclodextrin 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 cyclodextrin cavity. Such competition may also occur with endogenous substances existing at the absorption site. The displacement of the drug from the cyclodextrin cavity by exogenous and endogenous substances at the absorption site is responsible for acceleration of the drug absorption.[113] For instance, the overall process of drug absorption from the solid complex in the presence of the competing agent is shown in Figure 5, where k_d is the dissolution rate constant, K_c is the stability constant of the complex of the drug with the cyclodextrin, K_i is the stability constant of the complex of the competing agent with the cyclodextrin, and k_a is the absorption rate constant of the drug. High dissolution rates and the relative stability of the complexes ($K_i > K_c$) favor a free drug which is readily available for absorption. By contrast, a free cyclodextrin after the dissociation of the complex removes some components from the membrane surface, thereby modifying the transport properties of the membranes and facilitating the drug absorption, especially for water-soluble drugs.[20] In this section, recent findings on enhanced drug absorption by hydrophilic cyclodextrins administered via different routes are described.

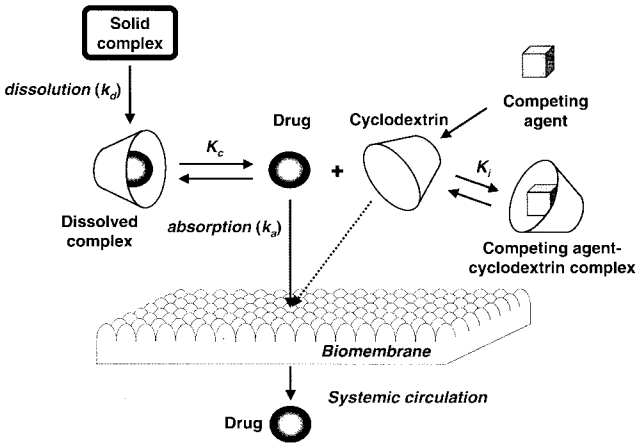


Figure 5. Schematic representation of the systemic absorption of drug from its cyclodextrin complex through biological membranes in the presence of the competing agent.

1. Oral Delivery

The commercial viability of cyclodextrin-based oral formulations has been established with the marketing of more than 10 products.[25] Hydrophilic cyclodextrins are particularly useful for improving stability, solubility, dissolution rate, and wettability of drugs through the formation of inclusion complexes.[20] The cyclodextrins 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 tracts. Recent studies have shown that multicomponent complexation of ketoconazole, an imidazole antifungal agent, with β-cyclodextrin and acids is superior to a binary complex of the drug with β-cyclodextrin in respect to oral absorption enhancement.[114,115] Other studies have demonstrated that a combination of α-cyclodextrin with citric acid is effective to prevent gel formation of cefotiam hexetil hydrochloride, an orally active antibacterial agent, promoting the dissolution rate and improving the oral bioavailability.[116] The potential use of hydrophilic cyclodextrins in developing the rate- or time-controlled oral formulations will be discussed in detail in section VI.A.

Rapidly dissolving complexes of drugs with hydrophilic cyclodextrins 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. A water-soluble complex of digoxin with γ-cyclodextrin can be formulated into a sublingual tablet to enhance bioavailability and to avoid acid hydrolysis of the drug by the gastric juices.[117] Other illuminating results were obtained for the sublingual administration of tablets containing complexes of steroids with cyclodextrins.[117−123] Hydrophilic cyclodextrins including 2-hydroxypropyl-β-cyclodextrin and β-cyclodextrin polymer supported the absorption of testosterone from the oral cavity and not from the gastrointestinal tracts; these solubilizers neither enter nor damage the oral tissues.[118] Inherently, the blood level of endogenous testosterone rises a few times a day in episodes lasting approximately 1 h. Such pulsatile release of testosterone can be imitated by the sublingual administration of its 2-hydroxypropyl-β-cyclodextrin complex, giving the desired pharmacological effects.[120] This formulation may be a useful addition to the currently available injectable and transdermal delivery systems for the treatment of hypogonadal men, which may be especially suitable for treatment of boys with delayed puberty and older men with an androgen deficiency.[122]

2. Rectal Delivery

The release of drugs from suppository bases is one of the important factors in the rectal absorption of the drugs, since the rectal fluid is small in volume and viscous compared to gastrointestinal fluid. In general, hydrophilic cyclodextrins enhance the release of poorly water-soluble drugs from oleaginous suppository bases because of the lesser interaction of the resultant complexes with the vehicles. The complexation of lipophilic drugs with hydrophilic cyclodextrins makes them insoluble in hydrophobic vehicles, the complex existing as well-dispersed fine particles in the vehicles. This manipulation not only enhances drug dissolution at an interface between the molten bases and the surrounding fluid but also inhibits the reverse diffusion of the drugs into the vehicles. In comparison with the parent cyclodextrins, the methylated cyclodextrins significantly enhance the rectal absorption of hydrophobic drugs such as flurbiprofen, an antiinflammatory agent,[124] carmofur,[125] and biphenylylacetic acid, an antiinflammatory agent,[39] from the oleaginous suppository. The superior effect of the methylated cyclodextrins can be explained by the faster release of the drugs together with the lowering of the affinity of the complexed drugs to the oleaginous suppository base.

2-Hydroxypropyl-β-cyclodextrin is particularly useful for improving the rectal absorption of the above drugs from oleaginous bases. The most striking effect of 2-hydroxypropyl-β-cyclodextrin was obtained for the enhancement of rectal absorption of antiinflammatory ethyl 4-biphenylyl acetate, a lipophilic prodrug of biphenylylacetic acid.[70,126] The relative potency of β-cyclodextrins in enhancing the dissolution rate of ethyl 4-biphenylyl acetate in water and reducing the binding affinity of the drug to the vehicle was β-cyclodextrin complex < 2-hydroxypropyl-β-cyclodextrin complex < heptakis(2,6-di-*O*-methyl)-β-cyclodextrin complex, which clearly fits the sequence of magnitude of stability constants of the complexes. However, the rectal absorption of ethyl 4-biphenylyl acetate was enhanced in the order of β-cyclodextrin complex < heptakis(2,6-di-*O*-methyl)-β-cyclodextrin complex < 2-hydroxypropyl-β-cyclodextrin complex after single and multiple administrations of suppositories containing the complexes in rats. The enhancement of rectal absorption of ethyl 4-biphenylyl acetate in vivo can be explained by the fact that 2-hydroxypropyl-β-cyclodextrin increases the release rate of ethyl 4-biphenylylacetate from the vehicle and stabilizes it in the rectal lumen, because the prodrug is more absorbable than the parent drug. The rather small enhancing effect of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin on the release rate of ethyl 4-biphenylylacetate than 2-hydroxypropyl-β-cyclodextrin is ascribable to the considerable dissociation of the complex in the vehicle together with increased viscosity of the suppository base, since heptakis(2,6-di-*O*-methyl)-β-cyclodextrin is more surface-active and oil-soluble than 2-hydroxypropyl-β-cyclodextrin. Of the three β-cyclodextrins tested, 2-hydroxypropyl-β-cyclodextrin had the highest potential to improve rectal absorption of ethyl 4-biphenylylacetate.

There is a great clinical need for the development of long-active types of rectal preparations of a potent opioid, morphine, in the treatment of intractable chronic pain in advanced cancer patients. However, the prolonged release of the opioid from the suppository is limited, showing a wide inter-individual variation of rectal bioavailability. Some hydrophilic cyclodextrins enhanced the rectal absorption of morphine from the hollow-type oleaginous suppository in rabbits.[127] In this case, the cyclodextrins did not alter the release rate of morphine from the vehicle, while they did however increase the mucosal membrane permeability to morphine. In addition, α-cyclodextrin reduced the glucuronidation of morphine during the passage through the rectal mucosa, probably through restricting the formation of a catalytic complex of morphine with glucuronyltransferase rather than because of the enzyme saturation.[128]

In combination with a viscosity-enhancing polysaccharide, xanthan gum, α-cyclodextrin reduced the first-pass metabolism of morphine in the rectal mucosa and by the liver and improved the apparent bioavailability of the opioid about 4-fold. From the observation of the distribution behavior of suppositories in rabbit rectum and colon after the rectal administration, xanthan gum was found to prevent the upward spread of the drug in the rectum.[127] This fact suggests that the absorption site for morphine should be limited at the lower part of the rectum to provide the elimination of the first-pass metabolism of morphine in the liver. In addition, gross and microscopic observations indicated that this preparation was less irritating to rectal mucosa. From the points of view of both safety and efficacy, therefore, this retentive opioid preparation seems to have an excellent therapeutic potential for the treatment of

severe malignant cancer pain, offering an improvement of the quality of life.

3. Nasal Delivery

Pulsatile release of steroids can be imitated by the nasal administration of water-soluble cyclodextrin complexes,[129−131] which may provide some desired pharmacological profiles as demonstrated in sublingual administration of a rapidly dissolving complex of steroids with 2-hydroxypropyl-β-cyclodextrin. Nasal preparations must be critically evaluated for their possible effect on the nasal mucociliary functions, which are known to defend the respiratory tract against dust, allergens, and bacteria. In the case of the nasal preparations containing the complexes of steroids with cyclodextrins, the effects of the cyclodextrins on the nasal epithelial membranes seem to be of minor importance for absorption enhancement, because the cyclodextrins would lose their abilities to interact with the membranes, when their cavities are occupied by steroids.[132]

The extent of systemic availability of morphine after the nasal administration of morphine hydrochloride in solution was much greater than those when the opioid was given orally in solution and rectally in suppository form in rats.[133] The ratio of area under the plasma level−time curve of an intact morphine to its main metabolite, morphine-3-glucuronide, via nasal administration was about twice those for both oral and rectal administrations and almost equivalent to that for the intravenous administration. Furthermore, higher levels of morphine in the cerebrospinal fluid were attained after the nasal administration compared with the oral and rectal routes. The nasal administration of morphine produced a dose-dependent analgesic response, as measured by the hot-plate method in rats. The efficacy of morphine to block the hot-plate response increased in the following order: oral < nasal < subcutaneous route. The scanning electron microscopic observations and membrane permeability studies using a transport marker, 6-carboxyfluorescein, revealed that morphine neither induced noticeable changes in surface morphology of the nasal mucosa nor affected the nasal membrane permeability to the transport marker. Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin significantly enhanced the rate of nasal absorption of morphine by facilitating the nasal epithelial permeability and consequently increased the entry of the opioid into the cerebrospinal fluid. In contrast, 2-hydroxypropyl-γ-cyclodextrin sustained the plasma levels of morphine, probably through the formation of a complex that was less permeable through membranes. Thus, a proper use of the cyclodextrins in nasal morphine preparations may provide adequate analgesia for both acute and chronic pain states, thus offering an improvement in patient comfort.[134]

A nasal spray containing estradiol solubilized in heptakis(2,6-di-*O*-methyl)-β-cyclodextrin was effective in the treatment of symptoms of estrogen deficiency in bilateral oophorectomized women, and the twice daily administration of this formulation over a period of 6 months was well tolerated by the patients.[135,136] Another illuminating result was obtained for the nasal administration of a drug with 2-hydroxypropyl-β-cyclodextrin, which was effective in suppressing colds in human volunteers challenged with rhinovirus type 9.[137] In this nasal spray, 2-hydroxypropyl-β-cyclodextrin was used as a solubilizer at doses of 2400 mg for 4 days and it caused no significant changes in hematological and biological measures in human volunteers. The potential of cyclodextrins to improve the pulmonary delivery of drugs has been also evaluated.[138,139]

4. Ocular Delivery

Possible advantages in ophthalmic use of cyclodextrins are the increase in solubility and/or stability and avoidance of incompatibilities of drugs, such as irritation and discomfort.[140] One of the prerequisites for a new vehicle to be used in ophthalmic preparations is that it is not irritating to the ocular surface, because irritation causes reflex tearing and blinking, which result in a fast washout of the instilled drug. Hydrophilic cyclodextrins, especially 2-hydroxypropyl-β-cyclodextrin and sulfobutyl-β-cyclodextrin, have been shown to be nontoxic to the eye and are well tolerated in aqueous eye drop formulations.[141,142]

The major problem with eye drops is its inability to sustain high local concentrations of drugs. The administration of ophthalmic drugs in gels and in polymer matrixes has been shown to increase the contact time of the drugs with the cornea, a situation which increases their ocular bioavailability. However, patient acceptance of such delivery systems is unsatisfactory. Conversely, eye drops with low viscosity appear to be the most acceptable delivery form of ophthalmic drugs. Hydrophilic cyclodextrins do not penetrate tight biological barriers such as the eye cornea but enhance the ocular bioavailability of lipophilic drugs by keeping the drugs in solution and increasing their availability at the surface of the corneal barrier.[143−148] For instance, the inclusion complex of dexamethasone acetate with 2-hydroxypropyl-β-cyclodextrin can be made as an ophthalmic solution. Although the volume of the precorneal fluid is too small to cause any significant dissociation of the complex, some endogenous lipids may displace the drug from the complex, thereby increasing the free drug level in the tear film and at the corneal surface. 2-Hydroxypropyl-β-cyclodextrin may further enhance the ocular bioavailability of dexamethasone acetate by inhibiting its bioconversion to dexamethasone, which shows lesser corneal permeability than the parent drug.[103] Recent studies have demonstrated that water-soluble polymers such as hydroxypropylmethylcellulose and polypyrroridone stabilize the complex of dexamethasone with 2-hydroxypropyl-β-cyclodextrin probably through the formation of a ternary complex, a situation which increases the drug solubility in aqueous eye drop and hence enhances the drug permeation into the aqueous humor in humans.[144] Also, a combination of 2-hydroxypropyl-β-cyclodextrin with hydroxypropylmethylcellulose can be used for improving the topical delivery of carbonic anhydrase inhibitors to the eyes.[92]

5. Dermal Delivery

Cyclodextrins have a significant safety margin in dermal application[149] and can be used to optimize the transdermal delivery of drugs intended either for local or systemic use.[150] Cyclodextrins improve the solubility and/or stability of drugs in the topical preparations,[49,151] to enhance the transdermal absorption of drugs,[91,152] to sustain the drug release from the vehicle,[153] and to avoid undesirable side effects associated with dermally applied drugs.[149,154]

A suitable vehicle must be selected so that cyclodextrins fully exert their functions. For instance, the in vitro release rate of corticosteroids from water-containing ointments (hydrophilic, absorptive, or polyacrylic base) is markedly increased by hydrophilic cyclodextrins, whereas in other ointments (a fatty alcohol propylene glycol or macrogol base) the cyclodextrins retard the drug release. The enhancement of drug release can be ascribed to an increase in solubility, diffusibility, and concentration of the drug in the aqueous phase of the ointment through water-soluble complex formation.[155,156] In ointments, as with suppositories, the drug in its cyclodextrin complex may be displaced by some components of the ointment, depending on the magnitude of the stability constant of the complex.[157] Thus, an optimized release of the drug from the preparation containing its cyclodextrin complex may be obtained by using a vehicle in which the complex is barely dissociated and maintains a high thermodynamic activity.

Cyclodextrins may interact with some components of the skin.[156,158−161] Differential scanning calorimetric studies have shown that heptakis(2,6-di-*O*-methyl)-β-cyclodextrin affected the endothermic transition of an isolated human stratum corneum, while no noticeable changes were observed for the stratum corneum treated with 2-hydroxypropyl-β-cyclodextrin.[159] Other studies demonstrated that randomly methylated β-cyclodextrin extracted all the major lipid classes from an isolated stratum corneum of hairless rats and reduced the barrier function of the skin, while 2-hydroxypropyl-β-cyclodextrin had limited specificity for cholesterol and triglycerides, and to a small extent, cholesterol esters.[160] Changes in barrier function of the skin induced by cyclodextrins may contribute in part to the enhancement of drug absorption. In such a case, particular attention should be directed toward possible irritation effects of the cyclodextrins on the skin. For example, the parent cyclodextrins at sufficiently higher concentrations caused skin irritation in guinea pigs in the order of γ- < α- < β-cyclodextrin, a result which depends largely on their abilities to extract lipids from the skin.[162]

In general, hydrophilic cyclodextrins and their complexes are supposedly absorbed through the skin only with considerable difficulty.[154] This is also the case in most other membranes. For instance, when heptakis(2,6-di-*O*-methyl)-β-cyclodextrin in 5% (w/v) methylcellulose solution was applied to the skin of rats, it was poorly absorbed transdermally and any intact heptakis(2,6-di-*O*-methyl)-β-cyclodextrin absorbed was quickly removed via the kidneys.[163] Similarly, when 2-hydroxypropyl-β-cyclodextrin in an aqueous solution was applied to the skin of hairless mice, its percutaneous absorption was extremely low at ~0.02% of the amount applied 24 h after topical application. Under the same condition, the amount of 2-hydroxypropyl-β-cyclodextrin absorbed from the tape-stripped skin was ~24%, suggesting that the stratum corneum acts as a barrier to the penetration of 2-hydroxypropyl-β-cyclodextrin through the skin.[164]

In contrast, when cyclodextrins are applied under the occlusive-dressing conditions and/or by using vehicles containing absorption-promoting agents, they are able to permeate the skin and exert some effects even there.[154] When the hydrophilic ointment containing ethyl 4-biphenylylacetate or its cyclodextrin complexes is applied to the skin of rats, the release of ethyl 4-biphenylylacetate from the ointment into the skin was enhanced by heptakis(2,6-di-*O*-methyl)-β-cyclodextrin or 2-hydroxypropyl-β-cyclodextrin, while β-cyclodextrin had no appreciable effect.[165−167] In addition, the β-cyclodextrins assisted the bioconversion of ethyl 4-biphenylylacetate to biphenylylacetic acid in the skin and consequently facilitated the delivery of active biphenylylacetic acid to subcutaneous tissues, where its action is most desired. The slow diffusion of ethyl 4-biphenylylacetate solubilized in 2-hydroxypropyl-β-cyclodextrin through the stratum corneum, together with the vehicle effect, could make the prodrug more susceptible to the metabolic process that is active in the epidermis, eventually leading to the facilitated activation of the prodrug.[168] In the model of carrageenan-induced acute oedema in rat paw, the inflammation was inhibited by the pretreatment with ointments containing complexes of ethyl 4-biphenylylacetate with the cyclodextrins: the efficacy order was the drug alone ≈ β-cyclodextrin complex < heptakis(2,6-di-*O*-methyl)-β-cyclodextrin complex ≈ 2-hydroxypropyl-β-cyclodextrin complex.[165]

Transdermal delivery of prostaglandin E_1 as an alternative to parenteral injections has engendered much recent interest in the treatment of peripheral vascular disorders. Since prostaglandin E_1 is chemically unstable and poorly permeable into the skin, a topical preparation capable of overcoming these problems needs to be devised to realize the full therapeutic potential of prostaglandin E_1. 6-*O*-(Carboxymethyl)-*O*-ethyl-β-cyclodextrin is found to markedly improve the chemical stability of prostaglandin E_1 in aqueous solution and ointments.[100] Topical application of an ointment containing the complex of prostaglandin E_1 with 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin supplemented with a penetration enhancer, 1-[2-(decylthio)ethyl]azacyclopentane-2-one, onto the skin of hairless mice resulted in a prominent increase in the cutaneous blood flow due to the vasodilating action of prostaglandin E_1.[48,49] Figure 6 shows changes in blood flow over time in rabbit's ears after topical application of prostaglandin E_1 or its cyclodextrin complexes in a fatty alcohol propylene glycol ointment supplemented with the penetration enhancer.[169] The prostaglandin E_1 ointments containing the penetration enhancer showed a marked increase in the blood flow; the vasodilation due to prostaglandin E_1 was limited to the area where the

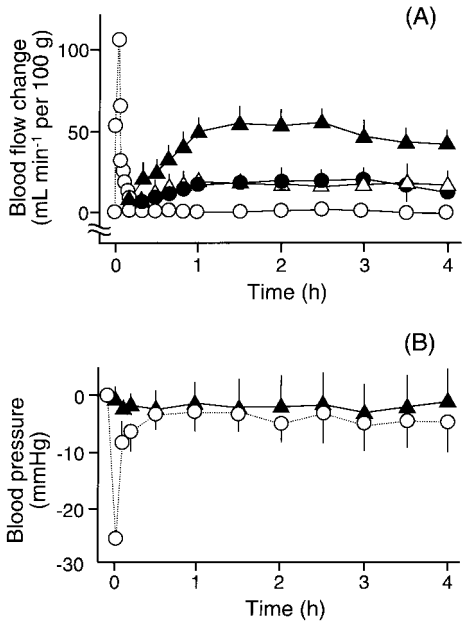


Figure 6. Changes in (A) regional blood flow and (B) femoral arterial blood pressure after topical application of fatty alcohol propylene glycol ointments (500 mg) containing prostaglandin E_1 and its cyclodextrin complexes (equivalent to prostaglandin E_1 at 0.01%, w/w) supplemented with 1-[2-(decylthio)ethyl]azacyclopentane-2-one (3% w/w) applied to rabbit's ears, compared with those after intravenous administration: ○, prostaglandin E_1 alone; ●, prostaglandin E_1 with 1-[2-(decylthio)ethyl]azacyclopentane-2-one; △, β-cyclodextrin complex with 1-[2-(decylthio)-ethyl]azacyclopentane-2-one; ▲, 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin complex with 1-[2-(decylthio)ethyl]-azacyclopentane-2-one. Each value represents the mean ± standard error of three or four rabbits. The dotted line represents the change in the blood flow and pressure after the intravenous administration of prostaglandin E_1 at a dose of 50 μg/body.

ointments were applied. The onset of action was within minutes of the application and the increase in the blood flow was sustained for at least 5 h, while no significant change was observed on the systemic arterial pressure or heart rate. In sharp contrast, an intravenous administration of prostaglandin E_1 at the same dose used in the topical preparation showed only transient rise in the blood flow, probably due to the rapid inactivation of prostaglandin E_1 in the lung. The vasodilating potencies of the prostaglandin E_1 ointments increased in the order of prostaglandin E_1 alone $\approx$ β-cyclodextrin complex < 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin complex. The combination of 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin and the penetration enhancer enhanced the percutaneous penetration of prostaglandin E_1 in a synergistic manner; 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin assisted the release of the penetration enhancer from the ointment base and its entry into the skin, which may facilitate the percutaneous penetration of prostaglandin E_1. Furthermore, this combination suppressed the bioconversion of prostaglandin E_1 to give less pharmacologically active metabolites during the passage through the skin, a situation delivering intact prostaglandin E_1 more effectively to the site of action. Prostaglandin E_1 and its cyclodextrin complexes, when supplemented with the penetration enhancer, significantly protected rabbits against the vascular occlusive sequelae induced with sodium laurate in the ear of rabbits. In particular, the ointment containing the complex of prostaglandin E_1 with 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin supplemented with the penetration enhancer showed the most prominent inhibitory effect on the progress of lesions; only regional discoloration was observed on day 7 after the injection of laurate. The inhibitory effect of prostaglandin E_1 ointments was consistent with the sequence of magnitude of their vasodilating actions. The preliminary tests for skin compatibility of prostaglandin E_1 ointments suggested no serious obstacles for their safe use. Therefore, a topical application of prostaglandin E_1 may be a rational choice for minimizing systemic side effects and patient compliance for long-term therapy.

D. Alleviation of Local and Systemic Toxicity

1. Reduction of Local Irritancy

The molecular entrapment of a drug into the cyclodextrin cavity may prevent direct contact of the drug with biological surfaces, and both the drug entry into the cells of nontargeted tissues and local irritation are thus decreased. In drug delivery of that type, the complex of a drug with cyclodextrin eventually dissociates into its components in a manner which depends on the magnitude of the respective stability constant, and thus there is no drastic loss of the therapeutic benefits of the drug. Therefore, cyclodextrins act as waferlike carriers which decrease the drug-induced local tissue damage at the administration site and then deliver the drug close to the desired site.

The lysis of isolated erythrocytes induced in vitro with drugs has been generally used to predict the local tissue irritancy of the drugs in vivo, allowing rapid screening of developing formulations at an earlier stage and reducing the need for time-consuming and costly animal studies. Cyclodextrins can protect erythrocytes against the hemolysis induced by various drugs including neuroleptics,[77,170] antiinflammatory drugs,[171] antibiotics,[172] etc. This protection is probably due to the reduction in effective concentrations of drugs in contact with the membrane, rather than the direct stabilizing effects on the membrane.[173]

Cyclodextrins alleviate muscular tissue damage following the intramuscular injection of drugs.[170−172,174,175] The protective effects of cyclodextrins may be attributed mainly to the poor affinity of the hydrophilic complexes of the drugs for the sarcolemmal membranes of muscle fibers, a situation expected from the results of in vitro hemolysis studies. For example, the intramuscular administration of chlorpromazine hydrochloride in the absence and presence of β-cyclodextrin derivatives to rabbits resulted in similar pharmacokinetic and pharmacodynamic profiles[176,177] but dramatically different histopathological presentations at the injection site and blood chemistry values. The release of the intracellular marker enzyme creatine phosphokinase from skeletal muscles into systemic circulation is a reliable

measure for estimating muscular irritation on injection. β-Cyclodextrin derivatives reduced the chlorpromazine-induced muscular damage and elevation of creatine phosphokinase activity in serum in the order of 2-hydroxypropyl-β-cyclodextrin < β-cyclodextrin < sulfobutyl-β-cyclodextrin, which clearly fits the sequence of magnitude of stability constants of the complexes of chlorpromazine with the β-cyclodextrin derivatives.[178] In particular, the changes in serum creatine phosphokinase activity from the sulfobutyl-β-cyclodextrin complex were almost identical to those from normal saline. Given the intrinsic intramuscular safety of the hydrophilic cyclodextrins and other potential advantages, several examples of their use in replacing irritating excipients in parenteral formulations have been published.[179−182]

Cyclodextrins diminish the ulcerogenic potency of several acidic antiinflammatory drugs[183−186] and mask their disgusting smells and tastes[183,188] when they are administered orally. The probable explanation for this observation is that the cyclodextrin complex dissolves faster and shows an accelerated absorption and thereby prevents the direct contact of irritative crystalline drug with the stomach wall.[183] A similar protection by hydrophilic cyclodextrins after rectal and ocular administrations of drugs was also described.[70,188−192] For example, 2-hydroxypropyl-β-cyclodextrin significantly reduced the irritation of rectal mucosa in rats caused by biphenylylacetic acid, both for single and multiple administrations of the complex of ethyl 4-biphenylylacetate with 2-hydroxypropyl-β-cyclodextrin in oleaginous suppositories.[70] More dramatic effect of cyclodextrins in decreasing ophthalmic irritation has been shown in a recent series of papers.[189−192] Pilocarpine is used topically for controlling the elevated intraocular pressure associated with glaucoma, but its ocular bioavailability is low due to the rapid loss from precorneal area. A bis(pilocarpic acid) diester prodrug showed improved ocular absorption of the parent drug and prolonged duration of action, but unfortunately also strong eye irritation was observed which may hinder its clinical usefulness. 2-Hydroxypropyl-β-cyclodextrin and sulfobutyl-β-cyclodextrin decreased the ophthalmic irritancy of the prodrug with no decrease in its miotic effectiveness.

Cutaneous contact photosensitization, due to phenothiazine neuroleptics, is known to cause severe problems to patients being treated with prolonged and high doses, to workers in manufacturing, to medical personnel, and to others who handle these drugs. Such unintentional exposure to these drugs has almost disappeared since the introduction of modified dosage forms such as the coated tablets and dispensable medication cups. However, this problem has not been solved satisfactorily for the injectable formulations. Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin significantly reduced the photosensitized skin irritation caused by chlorpromazine in guinea pigs according to the gross and histological examination.[154] Upon its binding to heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, the entry of chlorpromazine into the skin was decreased; furthermore, the photochemical reactivity of the drug was altered[193] and combination of these factors may lead to the observed desensitization. When chlorpromazine was photoirradiated with heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, high yields of promazine, which is less toxic than chlorpromazine, were produced. In addition, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin suppresses the formation of numerous oxidation and polymerization photoproducts which are responsible for the chlorpromazine-photosensitized skin reaction.[194] β-Cyclodextrins also decreased the photoinduced free-radical production from chlorpromazine,[154] the photodecarboxylation of benoxaprofen, an antiinflammatory agent,[195] and photodimerization of protriptyline, a tricyclic antidepressant,[196] resulting in the reduction of phototoxicity. Similarly, the β-cyclodextrin complexation is useful for reducing skin irritation induced with *all-trans*-retinoic acid-containing gel, which is successfully used in the treatment of psoriasis and acne.[197] Other studies have shown that a combination of 2-hydroxypropyl-β-cyclodextrin and oleic acid in propylene glycol enhanced the percutaneous absorption of isosorbide dinitrate, a vasodilator, and reduced skin reactions such as erythema and edema.[198]

2. Systemic Detoxication

An early study has demonstrated that the addition of β-cyclodextrin to dialysis fluids accelerated the removal of phenobarbital by peritoneal dialysis, thereby proving effective in the treatment of drug overdose.[199] A retinal−dextran conjugate solubilized by β-cyclodextrin was reported to be less cytotoxic and retained the ability to inhibit the growth of cancer cells.[200] 2-Hydroxypropyl-β-cyclodextrin serves as a benign vehicle for the delivery of drugs which are directed at sites in the central nervous system.[201,202] When opioids were administered intrathecally in rats, 2-hydroxypropyl-β-cyclodextrin prolonged the duration of analgesia and reduced the supraspinal side effects such as the incidence of catalepsy and blockade of the pinna reflex. 2-Hydroxypropyl-β-cyclodextrin appears to provide a slow release reservoir, diminishing the redistributional loss of spinally administered drug from the locus of desired effect to systemic circulation and extraspinal tissues.

Cyclodextrins can be used not only as an enabling excipient in pharmaceutical formulations but also as an artificial carrier for either exogenous or endogenous lipophiles in the body.[203,204] Some natural lipophiles are changed into toxic agents when the organism lacks the ability to transport and redistribute them properly by carrier proteins and their receptor systems. Furthermore, various exogenous lipophiles enter the body and accumulate in fat tissues, which do not metabolize them effectively. Consequently, these exogenous lipophiles may exert toxic action for a very prolonged period of time. In such cases cyclodextrins act as an artificial circulating carrier for the lipophiles in order to redistribute them in the extracellular space.

When heptakis(2,6-di-*O*-methyl)-β-cyclodextrin was administered parenterally to mice under retinoid-induced hypervitaminosis A, the survival rate of

poisoned animals was significantly improved.[203] This preliminary result was the impetus for the use of 2-hydroxypropyl-β-cyclodextrin to rescue a patient with familial hypervitaminosis A caused by overloading retinyl esters in the liver.[205] The patient received an intravenous infusion of 2-hydroxypropyl-β-cyclodextrin at a rate of 0.5 g/kg/day for ~4 days. During the infusion the serum levels of retinyl esters transiently increased and their urinary excretion was enhanced, indicating that 2-hydroxypropyl-β-cyclodextrin may assist the transfer of retinoids from the liver to circulating lipoproteins. There was 20−30% decrease in the circulating levels of total cholesterol during the infusion.

The lipid content and composition of some human tissues (e.g., intima of aorta and tendons) change with apparently disease-free aging. Similar changes occur with atherosclerosis, which involves deposits of excessive cholesterol and its esters mainly in the vascular system. On the cellular level, this excessive deposition is a result of receptor-mediated uptake of oxidized forms of low-density lipoproteins.[206] It may be possible to correct this situation by introducing into the circulation an alternate lipid carrier that would not be receptor directed and that would distribute lipids more evenly. Some positive results with this approach have been obtained with particulate preparations, such as liposomes and emulsions.[207] Another strategy is to use biocompatible molecular solubilizers, such as 2-hydroxypropylated cyclodextrins.

As described in section II.B.1., the in vitro study with human erythrocytes has demonstrated that β-cyclodextrin extracted cholesterol from the cell membranes into a new compartment located in the aqueous phase which could equilibrate rapidly with additional erythrocytes.[59] This process could be maintained by a thermodynamic equilibrium, where the cholesterol extraction may be triggered by the enhanced desorption of the lipid from the cell membranes into the aqueous compartment, rather than the transfer during transient contact between β-cyclodextrin and the cells in a similar manner as reported in the lipid transfer between lipid-containing vesicles.[208] Recent studies compared cellular cholesterol efflux mediated by either β-cyclodextrins (β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, and methylated β-cyclodextrins) or high-density lipoprotein, a physiological cholesterol acceptor.[209] β-Cyclodextrins induced the efflux of cholesterol from mouse L-cell fibroblasts with a major portion of cholesterol being released in the first 2 h of incubation. In contrast, high-density lipoprotein released cholesterol from the cells at a relatively constant rate during 8 h of incubation. Of particular interest is the observation that the efficacy of the cyclodextrins to induce the cellular cholesterol efflux is much greater than that of high-density lipoprotein, and cytotoxicity occurs only after prolonged incubation with the cyclodextrins but not during the first rapid phase of cholesterol efflux. More recent studies have demonstrated that the use of cyclodextrins as artificial shuttles, together with large unilamellar phospholipid vesicles as sinks, would be expected to enhance the clearance of vessel wall cholesterol to a greater extent than has been obtained with the sole use of either the cyclodextrins or the vesicles.[210]

In-vivo studies have shown that a single intravenous administration of 2-hydroxypropyl-β-cyclodextrin to rats or hereditary hyperlipidemic Watanabe rabbits slightly and temporarily decreased the level of total cholesterol in serum.[211,212] By contrast, single injections of 2-hydroxypropyl-β- and 2-hydroxypropyl-γ-cyclodextrins, both of which are less potent solubilizers of cholesterol,[213] had lesser effects. Repeated administration of 2-hydroxypropyl-β-cyclodextrin to the rabbits led to a gradual increase in total cholesterol in the circulation and eventually to a slight relief of atherosclerotic lesions in the thoracic aorta.[212] 2-Hydroxypropyl-β-cyclodextrin may act as a catalyst, shuttling cholesterol between compartments and enhancing rates of exchange. These rescue therapies with 2-hydroxypropyl-β-cyclodextrin are encouraging, but obviously the process will have to be further optimized before practical applications are possible. Furthermore, the speed of dissolution and the lack of irritation suggest that 2-hydroxypropyl-β-cyclodextrin may be useful during invasive procedures (e.g., angioplasty), when lipid debris may be released into the blood-stream.

Anion-exchange resins such as cholestyramine and colestipol are commonly prescribed as nonsystemic cholesterol-lowering drugs. However, the oral administration of these resins is frequently associated with constipation and triglyceride elevation. Occasionally it is difficult for patients to maintain a strict cholesterol-lowering regimen because of the resin's poor palatability. Dietary administration of β-cyclodextrin is an alternative approach for lowering blood cholesterol levels.[214−216] β-Cyclodextrin sequesters bile acids in the intestine through inclusion complexation[217,218] and prevents their reabsorption, thereby enhancing their fecal elimination. In contrast to cholestyramine, β-cyclodextrin is metabolized by the colonic microflora, partly releasing the sequestered bile acids for reabsorption. This process is accompanied by a significant production of short-chain fatty acids, which may counteract the up-regulation of bile acids and cholesterol biosynthesis. Although β-cyclodextrin is less potent at enhancing fecal excretion of bile acid than cholestyramine, a significant reduction of blood cholesterol is obtained only with this fermentable oligosaccharide. Therefore, β-cyclodextrin appears to exert the hypocholesterolemic effects via more complex physiological processes than cholestyramine.

Gentamicin, an aminoglycoside antibiotic, is widely used in the clinical treatment of Gram-negative infections, but its use is sometimes complicated by the development of drug-induced acute renal failure. Gentamicin is thought to interact with negatively charged phospholipids of lysosomal membranes in the proximal tubular cells, the interaction of which may lead eventually to lysosomal dysfunction, resulting in necrosis of the cells. Since some polyanions such as dextran sulfates are able to interact electrostatically with gentamicin and to reduce the drug's entry into the renal cortex, the effects of cyclodextrin

sulfates on development of rat renal dysfunction induced with gentamicin were studied.[78,219] Cyclodextrin sulfates, when given intraperitoneally, protected rats against renal impairment due to gentamicin, while the other hydrophilic cyclodextrins were ineffective. Since cyclodextrin sulfates did not reduce the total amount of gentamicin accumulated in the kidneys, this protection probably occurs through interfering with intracellular events leading from the drug accumulation to nephrotoxicity. When cyclodextrin sulfates were administered intraperitoneally to rats, a small but noticeable amount of cyclodextrin sulfates was recaptured and distributed into the renal microsomal fraction. In vitro studies indicate that cyclodextrin sulfates interact electrostatically with the amino groups of gentamicin and hence interfere with the interaction of the cationic drug with negative-charged lysosomal membranes in the proximal tubular cells, eventually leading to the protection of the kidneys against the drug-induced nephrotoxicity. In addition, cyclodextrin sulfates may enhance the renal regeneration derived from endogenous growth factors through the stabilization, which will be described in section VI.B.

IV. Cyclodextrin-Based Drug Delivery Systems

On the basis of the multifunctional characteristics of cyclodextrins, this section is mainly concerned with the latest applications of cyclodextrins in oral drug delivery, peptide and protein delivery, and site-specific delivery. In oral drug delivery, a simultaneous use of different cyclodextrins will enhance the function of each host molecule. Similarly, suitable combinations of molecular encapsulation with other carrier materials will be effective tools in the improvement of drug properties. Moreover, the peracylated or regioselectively acylated cyclodextrins may have broad applicability, and they can serve as novel hydrophobic or amphiphilic carriers of water-soluble drugs. In peptide and protein delivery, hydrophilic or ionizable cyclodextrins will enhance the drug absorption, while hydrophobic cyclodextrins will be useful in slow-release preparations. The biodegradation property of cyclodextrin may be useful as a colon-targeting carrier, and the cyclodextrin prodrugs will serve as a source of site-specific delivery of drugs to the colon.

A. Controlled Release in Oral Delivery

From the viewpoint of the optimization of pharmacotherapy, drug release should be controlled in accordance with the therapeutic purpose and the pharmacological properties of active substances. There has been a growing interest in developing the rate- or time-controlled type oral preparations, because an appropriate drug release from the dosage forms is of critical importance in realizing their therapeutic efficacy. Figure 7 shows the typical drug release−time profiles after oral administration. The plasma drug levels−time profiles can be mainly classified into two categories: rate-controlled type and time-controlled type (delayed release type). The rate-controlled type is further classified into three types, that is, immediate release, prolonged release, and modified release types. On the basis of this knowledge, various cyclodextrin derivatives have been used in order to modify drug release in oral preparations. The hydrophilic and hydrophobic cyclodextrins 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 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin. Moreover, a combination of cyclodextrins and other carrier materials is useful to optimize the release rate of drugs. The typical examples of the applications of cyclodextrin derivatives in the modified release and prolonged release formulations will be described next.

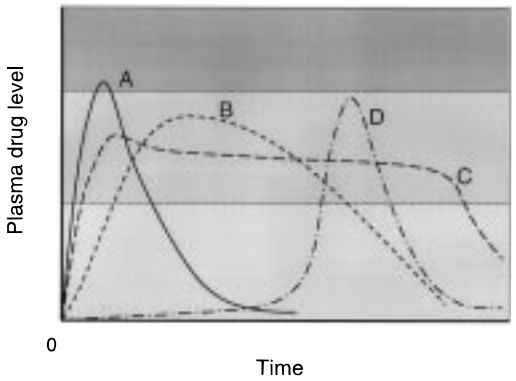


Figure 7. Typical drug release profiles following oral administration: A, immediate release; B, prolonged release; C, modified release; D, delayed release.

1. Immediate Release

Immediate release formulation of analgesics, antipyretics, coronary vasodilators, etc., is particularly useful in an 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 the drugs, various hydrophilic materials are used to attain the immediate release formulation. The hydrophilic cyclodextrins have been extensively applied to enhance the oral bioavailability of steroids, cardiac glycosides, nonsteroidal antiinflammatory drugs, barbiturates, antiepileptics, benzodiazepines, antidiabetics, vasodilators, etc.[7−15] These improvements are mainly ascribable to the increase in solubility and wettability of drugs through the formation of inclusion complexes.[20,21] The stabilizing effect of cyclodextrins on labile drugs is also responsible for the improvement of oral bioavailability. For example, γ-cyclodextrin complex decreases acid hydrolysis of cardiac glycosides, and hence improved the oral absorption of digoxin in dogs.[220] Recently, highly hydrophilic cyclodextrin derivatives, such as 2-hydroxypropyl-β-cyclodextrin,[221] 6-*O*-maltosyl-β-cyclodextrin,[222] and sulfobutyl-β-cyclodextrin[223] have been used to obtain an immediate release formulation, which is readily dissolved in the gastrointestinal tracts, promising an enhancement of oral bioavailability of poorly water-soluble drugs. 2-Hydroxypropyl-β-cyclodextrin is utilized to modify the physical properties of drugs in the solid state such as particle size, the polymorphic transition, and the conversion of crystalline to amorphous

or glassy state.[108,109] For example, the rapid dissolving forms of metastable and glassy states of nifedipine can be obtained by cooling the melts of a 1:1 physical mixture of 2-hydroxypropyl-β-cyclodextrin matrixes.[107]

Another approach is the use of amphiphilic cyclodextrins, such as β- or γ-cyclodextrin esterified on the secondary hydroxyl groups by alkyl chains (from C6 to C14). They are capable of forming self-assembled nanospheres which can be loaded with high amount 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, promising for the administration of poorly water-soluble drugs their rapid bioavailable.[224]

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, 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin was developed to exhibit pH dependent solubility for use in selective dissolution of the drug cyclodextrin complex.[45] The 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin displays limited solubility under the acidic conditions such as the stomach with the complex solubility increasing as the pH passes through the pK_a ($\approx$3.7) of the 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin. Then, the 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin complexes have been used in in-vitro and in-vivo studies with diltiazem, a calcium channel antagonist, and molsidomine, a peripheral vasodilator. The diltiazem studies were carried out in gastric acidity controlled fasting dogs with gastric pH controlled to less than two and greater than six. Diltiazem absorption was slower at high gastric acidity ($t_{max} = 4.0 \pm 0.5$ h) than at low gastric acidity ($t_{max} = 2.3 \pm 0.2$ h). The in-vitro release data measured using a pH changeable dissolution apparatus were in good agreement with the in-vivo data.[225] Molsidomine absorption from tablets containing 6-*O*-(carboxymethyl)-*O*-ethyl-β-cyclodextrin was studied in gastric acidity controlled dogs in the fasted and fed states. Under high gastric acidity the molsidomine absorption was significantly retarded relative to the low gastric acidity condition. The delayed absorption 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.[226]

3. Prolonged Release

Most of the slow-release preparations have been aimed at achieving the 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 efficacy, and avoiding the toxicity associated with the administration of a simple plain tablet. For this purpose, hydrophobic cyclodextrins such as alkylated and acylated derivatives are useful as slow-release carriers for water-soluble drugs. Among the alkylated cyclodextrins, heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin and heptakis-(2,3,6-tri-*O*-ethyl)-β-cyclodextrin were the first slow-release carriers to be used in conjunction with diltiazem[41,227] and isosorbide dinitrate[228] following oral administrations of the hydrophobic complexes to dogs. On the other hand, the peracylated cyclodextrins with medium alkyl chain lengths (C4−C5) are particularly useful as novel hydrophobic carriers, because of their multifunctional and bioadaptable properties.[19] They have broad applicability in various routes of administration: for example, the bioadhesive property of heptakis(2,3,6-tri-*O*-butanoyl)-β-cyclodextrin (C4) can be used in oral and transmucosal formulations, while the film-forming property of heptakis(2,3,6-tri-*O*-valeryl)-β-cyclodextrin (C5) is useful in transdermal preparations.[229] 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 the complexation with peracylated β-cyclodextrins in the decreasing order of their solubility, in particular by those longer than the butylated derivatives.[230] When the complexes were administered orally to beagle dogs, heptakis(2,3,6-tri-*O*-butanoyl)-β-cyclodextrin suppressed a peak plasma level of molsidomine and maintained a sufficient drug level for long periods, while a single use of other derivatives having shorter or longer chains than heptakis(2,3,6-tri-*O*-butanoyl)-β-cyclodextrin proved insufficient. A prolonged maintenance (at least 24 h) of higher and constant salbutamol levels in plasma, a bronchodilator, was also obtained after oral administration of the heptakis(2,3,6-tri-*O*-butanoyl)-β-cyclodextrin complex in dogs, where the plasma level of the major metabolite, salbutamol glucuronide, was significantly lower than that after administration of the drug alone.[231] This indicates that heptakis(2,3,6-tri-*O*-butanoyl)-β-cyclodextrin may be a useful carrier for orally administered water-soluble drugs, especially for drugs which are metabolized in the GI tracts. The superior sustaining effect exhibited with the heptakis(2,3,6-tri-*O*-butanoyl)-β-cyclodextrin may be a result of both increased hydrophobicity and mucoadhesive properties. Similarly, nanospheres formed by heptakis(2,3-di-*O*-hexanoyl)-β-cyclodextrin may possess bioadhesive properties on gastrointestinal mucosa.[224]

Moreover, a combined use of short- and long-chain peracylated β-cyclodextrins in appropriate molar ratio is effective to control the release rate of water-soluble drugs.[232] For example, the release rate of diltiazem decreased in the order of the increase in hydrophobicity of carrier materials. The change in release rate of the drug from the hydrophobic carriers

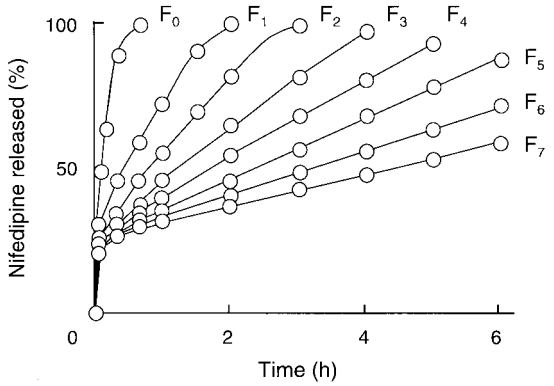


Figure 8. Release profiles of nifedipine (5 mg) from plain tablet (F_0) and seven double-layer tablets (F_1−F_7) in water at 37 °C.

was clearly reflected in the blood drug levels after oral administration of the tablets in dogs. Although the 1:2 heptakis(2,3,6-tri-*O*-acetyl)-*β*-cyclodextrin (C1) system maintained the plasma drug levels of over 30 ng/mL for at least 24 h, a combination of heptakis-(2,3,6-tri-*O*-acetyl)-*β*-cyclodextrin and heptakis(2,3,6-tri-*O*-octanoyl)-*β*-cyclodextrin (C8) gave a constant plasma drug level (20−40 ng/mL) for more than 48 h, with a significant increase in the extent of bioavailability.

4. Modified Release

The conventional formulation of nifedipine, a typical calcium-channel antagonist, must be dosed 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 problems, such as low oral bioavailability due to poor aqueous solubility and a decrease in dissolution rate during the storage due to the crystal growth.[106] Therefore, the release rate of nifedipine must be modified in order to obtain a more balanced oral bioavailability with prolonged therapeutic effect. Uekama and co-workers have recently developed a double-layer tablet, using 2-hydroxypropyl-*β*-cyclodextrin and pharmaceutical excipients, and evaluated the drug release behavior. An amorphous nifedipine powder prepared by spray-drying with 2-hydroxypropyl-*β*-cyclodextrin and a nonionic detergent HCO-60 was employed as a fast-release portion to attain an initial rapid dissolution and to prevent the crystal-growth during storage.[233] Hydroxypropylcelluloses with different viscosity grades were employed as a slow-release portion to provide an appropriate sustained release of poorly water-soluble nifedipine from the viscous matrices. Then, an optimal formulation of the double-layer tablet was surveyed by changing the mixing ratio of a fast-release portion and a slow-release portion (Figure 8). The in-vitro release rate of nifedipine from the double-layer tablet was little affected by pH of the medium and rotation speed of paddle even after the long-term storage in accelerated conditions (60 °C, 75% RH).[234] Among the seven formulations tested, the double-layer tablet (F3) consisting of 2-hydroxypropyl-*β*-cyclodextrin with 3% HCO-60/(hydroxypropylcellulose (low viscosity grade):hydroxypropylcellulose (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.[235] These facts suggest that a combination of 2-hydroxypropyl-*β*-cyclodextrin, HCO-60, and hydroxypropylcelluloses serves as a modified-release carrier of nifedipine and can be applied to the 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 an efficient bioavailability for such acidic drugs, a double-layer tableting was also useful, consisting of hydrophilic cyclodextrins as the fast-release portion and hydrophobic cellulose derivatives as the slow-release portion.[236] In the case of piretanide, the tablet consisting of the [heptakis(2,6-di-*O*-methyl)-*β*-cyclodextrin/(hydroxypropylcellulose/ethylcellulose)] system in the weight ratio [1/3(1/3)] provided a sufficient slow release of the drug over 8 h in a wide pH region following an initial rapid dissolution.

Recently, Okimoto and co-workers have developed a novel osmotic pump tablet for prednisolone, applying sulfobutyl-*β*-cyclodextrin which acts as a solubilizer and an osmotic agent.[237] Core tablets containing the drug and osmotic agents were film-coated with cellulose acetate. The in-vitro release studies revealed that prednisolone from the osmotic pump tablets with sulfobutyl-*β*-cyclodextrin was completely released, in contrast with those in 2-hydroxypropyl-*β*-cyclodextrin or sugars. The plasma drug profiles for the osmotic pump tablets following oral administration to dogs were retarded with the decreased in-vitro rate.

B. Peptide and Protein Delivery

Advances in biotechnology have accelerated the economical, large-scale production of therapeutically active peptide- and protein-based drugs used to combat poorly controlled diseases, making them more readily available for therapeutic use. This rapid progress in molecular biology, however, has not been matched by the progress in the formulation and development of delivery systems for peptide and protein drugs. There are considerable hurdles to be overcome before practical use can be made of therapeutic peptides and proteins because of chemical and enzymatic instability, poor absorption through biological membranes, rapid plasma clearance, peculiar dose−response curves, and immunogenicity. Many attempts have addressed these problems by chemical modifications or by coadministration of adjuvants to eliminate undesirable properties of peptides and proteins.[238] Cyclodextrin complexation seems to be an attractive alternative to these approaches.

1. Interaction of Cyclodextrins with Peptides and Proteins

Cyclodextrins can recognize not only the size and shape but also the chirality of amino acids.[239,240]

However, molecules of many peptides and proteins are too hydrophilic and bulky to be wholly included in the cyclodextrin cavity and the topological constraints of the peptide backbone may reduce the formation of inclusion complexes, thus their interaction with cyclodextrins could be only local; that is, accessible hydrophobic side chains may form inclusion complexes with cyclodextrins.[241,242] Such interaction possibly affects the overall three-dimensional structure of peptides and proteins or inhibits their intermolecular association and thus changes their chemical and biological properties.

A synthetic nonapeptide buserelin acetate, defined as pyroGlu-His-Trp-Ser-Tyr-D-Ser(*tert*-butyl)-Leu-Arg-Pro-ethylamide, is a highly potent agonist of luteinizing hormone-releasing hormone. Spectroscopic studies indicate that the aromatic side chains of buserelin acetate, L-tryptophan and L-tyrosine residues, are incorporated into the hydrophobic environment of the heptakis(2,6-di-*O*-methyl)-β-cyclodextrin cavity. Furthermore, ^{1}H- and ^{13}C-NMR spectroscopies suggest that in addition to the two aromatic side chains, a tertiary butyl D-serine residue is inserted into the heptakis(2,6-di-*O*-methyl)-β-cyclodextrin cavity from the secondary hydroxyl side. On the other hand, the continuous variation plots for the buserelin acetate−heptakis(2,6-di-*O*-methyl)-β-cyclodextrin system showed a 1:1 stoichiometry of the complex. Therefore, the complexation should be initiated by the inclusion of one of the three binding sites on the buserelin molecule into heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, which may in turn prevent the further access of the second cyclodextrin to the other binding sites, probably due to steric hindrance and/or conformational changes of the peptide. Consequently, the three buserelin acetate complexes with heptakis(2,6-di-*O*-methyl)-β-cyclodextrin at a 1:1 molar ratio with a difference in the binding site may coexist in the solution. This heterogeneity in the structure of the complex formed is consistent with the complexity in the near-ultraviolet circular dichroism spectrum, indicating that, upon binding to heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, the local environment around the aromatic groups in the peptide differs distinctly.[243]

Hydrophilic cyclodextrins affect the tertiary structure of recombinant human growth hormone in aqueous solution. The electrospray ionization mass spectrum of the growth hormone obtained in an acidic medium gave a broad charge distribution. The higher charge state distribution was observed for the growth hormone with 6-*O*-maltosyl-β-cyclodextrin, suggesting less compact conformation of the protein (Figure 9).[244] Furthermore, in the presence of 6-*O*-maltosyl-β-cyclodextrin, new signals were observed, which correspond to the 1:1 and 1:2 adducts of the ionized growth hormone with the cyclodextrin. Calorimetric studies for thermal unfolding of the growth hormone at a neutral pH demonstrated that the ratio of the van't Hoff enthalpy to the calorimetric enthalpy ($\Delta H_v/\Delta H_c$) was more than 1, indicating the aggregation of the protein during the unfolding process. The hydrophilic cyclodextrins increased the unfolding temperature for the growth hormone and decreased

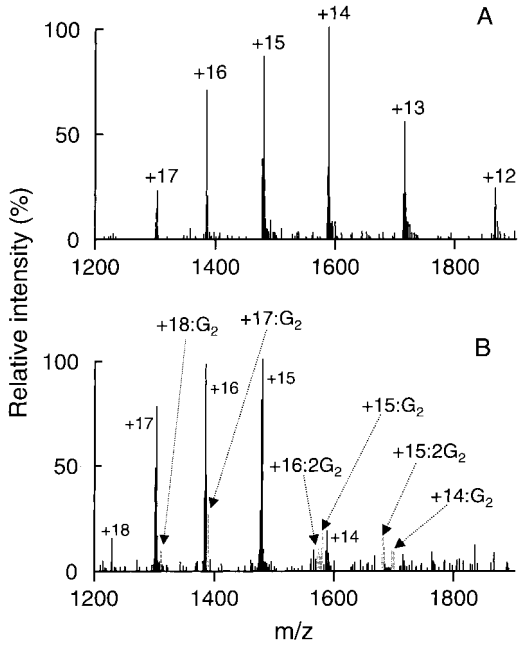


Figure 9. Electrospray mass spectra of human growth hormone (0.1 mM) in positive ion mode in the absence and presence of 6-*O*-maltosyl-β-cyclodextrin (10 mM) in water/methanol/acetic acid (49/49/2) solution (pH 3.0).

the $\Delta H_v/\Delta H_c$ ratio. These results suggest that the interaction of the hydrophilic cyclodextrins with accessible hydrophobic side chains in the growth hormone molecule leads to the less compact conformation of the protein and reduces its aggregation during the unfolding process.

β-Cyclodextrin is known to bind to the substrate binding domain of maltodextrin binding protein from *Escherichia coli* with the same stability order as those of the linear maltodextrins, although it is not a physiological ligand for that protein.[245] Other studies have shown that β-cyclodextrin binds to the starch-binding domain of *Aspergillus niger* glucoamylase but does not inhibit the enzyme activity, indicating that there is no interaction between the catalytic and the starch-binding domains.[246−249]

2. Hydrophilic Cyclodextrins as Solubilizers and Stabilizers

Cyclodextrins can be used to solubilize and stabilize various biomedically important peptides and proteins including growth hormones[250,251] interleukin-2,[250] monoclonal antibody MN12,[252] aspartame,[253] tumor necrosis factor,[254] β-amyloid peptide,[255] albumin,[256] γ-globulin,[256] lactate dehydrogenase,[257] etc. For instance, α-cyclodextrin increases the solubility of cyclosporin A, an immunosuppressive agent, in eye-drop form, and helps the drug to penetrate into the cornea with the least local toxicity.[258,259] Of the hydrophilic cyclodextrins tested, hexakis(2,6-di-*O*-methyl)-β-cyclodextrin is the most potent solubilizer for cyclosporin A and increases the extent of the oral bioavailability about 5-fold but does not affect its lymphatic transfer.[260,261]

Interactions of cyclodextrins with side chains on oligomeric peptides can dissociate the oligomers, especially if the complexation occurs at sites in the peptide−peptide interface. The propensity of insulin

to form reversible and irreversible aggregates in solution leads to complications in the development of long-term insulin therapeutic systems and limits the rate of subcutaneous absorptions, a process which is too slow to mimic the physiological plasma insulin profile at the time of meal consumption. These problems are further complicated by the tendency for insulin to adsorb on to the surfaces of containers and devices, perhaps by mechanisms similar to those inducing aggregation.[262] Some hydrophilic cyclodextrins, including 2-hydroxypropyl-β-cyclodextrin and 6-*O*-maltosyl-β-cyclodextrin, significantly inhibit the adsorption of insulin to hydrophobic surfaces of containers and its aggregation in neutral solutions.[263] 2-Hydroxypropyl-β-cyclodextrin is also found to prevent the shaking-induced formation of insoluble aggregates of insulin in neutral solutions.[264,265] Both β-cyclodextrins facilitated the permeation of insulin through the ultrafiltration membranes and increased the surface tension of insulin solutions. In the circular dichroism spectrum of insulin, the β-cyclodextrins increased the negative band intensity around 208 nm assigned to the α-helix structure of insulin while decreased that around 275 nm assigned to the antiparallel β-structure of insulin oligomers. These spectral changes are in close agreement with those observed when insulin aggregates are dissociated to monomer or lower-order aggregates. Hydrogen−deuterium (H/D) exchange measurements coupled with electrospray ionization mass spectrometry have shown that the exchange rate of insulin was rapid in 30% v/v acetic acid solution where the peptide is predominantly in a monomer state, and the rate was unchanged by the addition of 6-*O*-maltosyl-β-cyclodextrin. However, the exchange rate significantly slowed in pH 2.0 solution, where insulin is predominantly in a dimer state, and the rate increased with increasing 6-*O*-maltosyl-β-cyclodextrin concentrations, indicating that 6-*O*-maltosyl-β-cyclodextrin shifts the monomer−dimer equilibrium of insulin in favor of the dissociated form.[266]

Dilution microcalorimetric study indicates a sequential binding of cyclodextrins to at least two possible sites on the insulin monomer at acidic condition.[267] On the basis of two-dimensional ^{1}H NMR measurements, 6-*O*-maltosyl-β-cyclodextrin may include accessible hydrophobic amino acid residues of insulin such as phenylalanine and tyrosine at the N-terminal end (B1) and in the C-terminal region (B25 and B26) of the B-chain, these side chains having a high motional freedom, while the side chains in the α-helices are not significantly perturbed in the presence of 6-*O*-maltosyl-β-cyclodextrin.[268] Thus, 6-*O*-maltosyl-β-cyclodextrin should perturb the intermolecular hydrophobic contacts between aromatic side chains across the monomer−monomer interfaces, eventually leading to the inhibition of self-association of the peptide. By contrast, sulfobutyl-β-cyclodextrin showed varying effects on insulin aggregation, depending on the degree of substitution of the sulfobutyl group, i.e., the inhibition at relatively low substitution and acceleration at higher substitution. In such a case, the electrostatic interaction between the positive charges of insulin and the negative charges of the sulfonate group of sulfobutyl-β-cyclodextrin seems to be more of a factor than the inclusion effects. These results suggest that proper use of the hydrophilic cyclodextrins could be effective in designing rapid or long-acting insulin preparations.[269]

The process by which protein molecules achieve their native compact conformations is a subject of both fundamental and practical importance. In particular, the practical interest in the protein refolding problem stems from the fact that proteins are overproduced by genetically engineered cells in the form of cytoplasmic aggregates or inclusion bodies, in which the proteins are misfolded and therefore functionally inactive. Weak interactions of cyclodextrins with unfolded proteins may enhance the solubility of denaturated proteins by masking the exposed hydrophobic residues, thereby possibly assisting the refolding of the proteins. In this way cyclodextrins might act as small chaperone mimics in the protein folding process in cases where refolding is inhibited by poorly reversible aggregation or entanglement.[270,271] More efficient protein refolding is established when cyclodextrins are used in combination with detergents.[272−275] In the first step, the nonnative target protein is captured by a detergent under conditions that would normally lead to irreversible protein aggregation, in which the substrate protein cannot spontaneously refold from the detergent-complexed state. In the second step, removal of the detergent from the protein is triggered by the addition of a cyclodextrin, allowing the protein to refold.

3. Hydrophilic Cyclodextrins as Absorption Enhancers

The systemic delivery of peptide- and protein-based drugs via various mucosal routes is receiving extensive scrutiny as an alternative to the oral and parenteral routes. The transmucosal delivery has advantages of being noninversive and of bypassing gastrointestinal and hepatic clearances. Among them the peptide delivery through nasal mucosa seems to be most successful and practical; nasal sprays for some therapeutic peptides are already available commercially.[238] However, even with the intranasal route of delivery, the nasal epithelium presents both a physical and a metabolic barrier to the absorption of peptides and proteins. Therefore, the use of absorption-promoting agents is necessary to achieve sufficient intranasal absorption of most peptides and proteins. The potential of cyclodextrins, especially the methylated cyclodextrins, as nasal absorption enhancers has been demonstrated for luteinizing hormone-releasing hormone agonists,[276,277] insulin,[278−281] adrenocorticotropic hormone analogue,[282] calcitonin,[283] granulocyte colony-stimulating factor,[284] insulin-like growth factor-I,[285] etc. The absorption enhancement afforded by cyclodextrins can be attributed primarily to their ability to reduce the physical and/or metabolic barriers to these peptides and proteins.

The limited systemic bioavailability of peptides and proteins is partly due to the existence of a substantial enzymatic barrier in the epithelial cells. Cyclodextrins can protect peptides and proteins against

enzymatic as well as chemical degradation.[286,287] For example, cyclodextrins, especially 6-*O*-maltosyl-*β*-cyclodextrin, significantly inhibited the enzymatic degradation of buserelin acetate in rat nasal mucosa.[277] On the basis of the inclusion mode of buserelin acetate with cyclodextrins as described in section IV.B.1, they may protect buserelin acetate sterically from proteolytic enzymes, by including the hydrophobic side chains of the peptide within the cyclodextrin cavity, because these binding sites are located near the enzymatic cleavage sites of the peptide.[277] However, the possibility for the cyclodextrins to inactivate directly the proteolytic enzymes should not be totally dismissed.[280,288,289] For example, 6-*O*-maltosyl-*β*-cyclodextrin decelerated the hydrolysis of buserelin acetate catalyzed by α-chymotrypsin, a typical serine protease. On the basis of kinetic studies, this deceleration can be explained solely by a nonproductive encounter between a complex of the substrate with 6-*O*-maltosyl-*β*-cyclodextrin and the protease at relatively low cyclodextrin concentrations, while the direct inhibitory effect of 6-*O*-maltosyl-*β*-cyclodextrin on the proteolytic activity made a considerable contribution to the overall deceleration of the hydrolysis at higher cyclodextrin concentrations. Further insight into the direct interaction between 6-*O*-maltosyl-*β*-cyclodextrin and α-chymotrypsin was gained by differential scanning calorimetry. The thermodynamic parameters for α-chymotrypsin alone indicate the presence of intermediate states in the thermal unfolding of the protease, simultaneously accompanied by the autolysis. By contrast, a two-state thermal unfolding of α-chymotrypsin was observed in the presence of 6-*O*-maltosyl-*β*-cyclodextrin. A similar two-state denaturation of α-chymotrypsin is observed under the condition that the protease is no longer reactive. These results indicate that 6-*O*-maltosyl-*β*-cyclodextrin reduces the catalytic activity of α-chymotrypsin in such a way that the accessible hydrophobic side chains of the protease may be incorporated into the cyclodextrin cavity, a situation which should produce some localized distortion and/or steric hindrance near the catalytic site of the protease.[289]

Another potential barrier to the nasal absorption of peptides and proteins is the limitation in the size of hydrophilic pores through which they are thought to pass. As described in section II.B.1, the hydrophilic cyclodextrins can solubilize some specific lipids from biological membranes through the rapid and reversible formation of inclusion complexes, leading to an increase in the membrane permeability.[59] Cyclodextrins may affect nasal mucosal membranes in the same manner, thus allowing their extended use as adjuvants to improve the nasal absorption of poorly absorbable peptide and protein drugs. The lipid solubilization mediated by cyclodextrins may cause changes in transcellular processes, and these changes are believed to be transmitted to the paracellular region, which appears to be the most likely route for the transport of polypeptides.[280,290]

Nasal preparations must be critically evaluated for their possible effect on the nasal mucociliary functions, which are known to defend the respiratory tract against noxious inhaled materials such as dust, allergens, and bacteria. Since most of the enhancers including cyclodextrins may promote the systemic absorption of peptides and proteins by perturbing membrane integrity in a rather nonspecific manner, it is inevitable that varying extents of insult would occur to the mucosal tissue in intimate contact with the enhancers. When compared with other absorption-promoting agents and preservatives used commonly in nasal formulations, cyclodextrins exert a rather mild and reversible effect on the surface morphology of nasal mucosa and the ciliary beating.[291−293] To evaluate the nasal tissue tolerability to cyclodextrins, the release behaviors of several biochemical markers from nasal mucosa were measured using an in-situ recirculating perfusion technique in rats.[294] Five percent solutions of cyclodextrins released the biochemical markers from the nasal mucosa with the efficacy increasing in the order sulfobutyl-*β*-cyclodextrin ≤ 2-hydroxyethyl-*β*-cyclodextrin < 2-hydroxypropyl-*β*-cyclodextrin < heptakis-(3-mono-*O*-methyl)-*β*-cyclodextrin < heptakis(2,6-di-*O*-methyl)-*β*-cyclodextrin, a sequence which is almost proportional to their hemolytic activity or ciliotoxicity. Other studies using an in-vivo lavage technique have shown that the rat nasal mucotoxicity increased in the order 2-hydroxyethyl-*β*-cyclodextrin < randomly methylated *β*-cyclodextrin < heptakis(2,6-di-*O*-methyl)-*β*-cyclodextrin < sodium glycocholate < sodium taurodihydrofusidate < L-α-lysophosphatidylcholine < an nonionic detergent laureth-9.[295] This rank order correlates well with those observed in morphological as well as ciliotoxicity studies. On the basis of the results of these studies, the minimal concentration of heptakis(2,6-di-*O*-methyl)-*β*-cyclodextrin necessary to achieve substantial absorption enhancement in rats is considered to be ∼2%, showing only a mild effect on the nasal ciliary function. It should be also noted that the efficacy and safety of cyclodextrins differ largely between species[296−298] and is also greatly dependent upon the dosage form.[299−302]

In an attempt to design a more effective and less irritating nasal formulation, combinations of cyclodextrins with absorption enhancers have received increasing attention.[303−307] For instance, 2-hydroxypropyl-*β*-cyclodextrin solubilized the lipophilic penetration enhancer 1-[2-(decylthio)ethyl]azacyclopentane-2-one and potentiated its action at an appropriate combination ratio without causing severe local irritation on the nasal application.[308−310] When the concentration of the penetration enhancer was kept constant at 1% (w/v), the nasal membrane permeability of fluorescein isothiocyanate dextran with an average molecular mass of 4400 Da increased with a rise in the 2-hydroxypropyl-*β*-cyclodextrin concentration, reaching a maximum when just enough 2-hydroxypropyl-*β*-cyclodextrin (∼15%, w/v) was used to keep all the enhancer in solution. 2-Hydroxypropyl-*β*-cyclodextrin may potentiate the activity of the penetration enhancer by solubilizing, thus making it more available at the mucosal surface for subsequent penetration into the nasal epithelium, a site of action. Upon further addition of 2-hydroxypropyl-*β*-cyclodextrin beyond the critical concentration nec-

essary for complete solubilization of the penetration enhancer, the activity of the enhancer decreased. The excess amount of 2-hydroxypropyl-β-cyclodextrin in solution may reduce the free fraction of the penetration enhancer, which is in an equilibrium with the complexed form, and thereby reduce the thermodynamic activity of the enhancer at the mucosal surface.

The sole use of the penetration enhancer in emulsion showed the maximal enhancing effect on the nasal absorption of fluorescein isothiocyanate dextran with an average molecular mass of ~40 000. On the other hand, the combination of the penetration enhancer with 2-hydroxypropyl-β-cyclodextrin provided the prominent enhancement of the nasal absorption of fluorescein isothiocyanate dextrans with average molecular masses of less than ~20 000, compared with that obtained for the sole use of the enhancer. With increasing molecular masses of fluorescein isothiocyanate dextrans, the permeation enhancing effect of this combination decreased steeply. The rate of overall processes involving the dissociation of the penetration enhancer from its 2-hydroxypropyl-β-cyclodextrin complex and subsequent uptake of the free form of the enhancer into the nasal mucosa was much faster than that of the enhancer from oil droplets into the mucosa. Therefore, the rapid onset and short duration of the enhancing effect of this combination would be not enough to allow the slow diffusion of fluorescein isothiocyanate dextrans with higher molecular masses through the nasal mucosa.[311]

These results indicate that the penetration enhancer solubilized in 2-hydroxypropyl-β-cyclodextrin at the appropriate combination ratio could cause just enough and transient perturbation of the nasal mucosa to allow the absorption of the permeation marker, without widespread damage of the epithelium. The approach described here would be extended to the optimal use of other lipophilic absorption enhancers particularly in the environment of the mucosal absorption site.

4. Hydrophobic Cyclodextrins as Sustained-Release Carriers

Chronic treatment with peptide and protein drugs has disadvantages; the short biological half-lives of the drugs require long-term daily injection or frequent nasal application in order to maintain therapeutic concentration of the drugs. Therefore, attention has been directed toward the development of drug delivery systems with controlled-release features so as to realize their potential and efficacy. Several approaches have been proposed including the use of implants or injectable microcapsules of biodegradable copolymers or gel-forming agents.

Injectable oily suspensions of buserelin acetate with sustained-release feature can be obtained by using hydrophobic cyclodextrins such as heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin[43,312] and heptakis(2,3,6-tri-*O*-acetyl)-β-cyclodextrin and octakis(2,3,6-tri-*O*-acetyl)-γ-cyclodextrin.[313] The interfacial transfer of buserelin from the peanut oil suspension into the aqueous phase was significantly retarded by the hydrophobic cyclodextrins in the order of heptakis(2,3,6-tri-*O*-acetyl)-β-cyclodextrin < octakis(2,3,6-tri-*O*-acetyl)-γ-cyclodextrin < heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin. The drug release from a vehicle is influenced by various factors including drug−vehicle interactions, solubility, partition coefficient, and particle size of drug in the vehicle. Buserelin acetate was practically insoluble in peanut oil, and the solubility was only slightly increased by the complexation with heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin or the peracethylated cyclodextrins. In contrast, the solubility of the cyclodextrins in the vehicle increased in the order of heptakis(2,3,6-tri-*O*-acetyl)-β-cyclodextrin < octakis(2,3,6-tri-*O*-acetyl)-γ-cyclodextrin < heptakis-(2,6-di-*O*-ethyl)-β-cyclodextrin, corresponding with the retardation order of buserelin release. The above results suggest that the drug might be dispersed within an oily matrix through a weak interaction of the drug with the cyclodextrins.

A single subcutaneous injection of the oily suspension of buserelin acetate containing heptakis(2,3,6-tri-*O*-acetyl)-β-cyclodextrin, octakis(2,3,6-tri-*O*-acetyl)-γ-cyclodextrin and heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin in rats provided retardation of plasma levels of buserelin, with giving 25, 39 and 70 times longer mean residence time, respectively, than that of the drug alone. Simultaneously with the suppression of plasma testosterone to castrate level, the antigonadal effect of buserelin continued for 1, 2, and 4 weeks and a significant weight reduction in genital organs was observed. For example, when the heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin complex was administered subcutaneously to rats, the weight of the genital organs decreased on week 1 due to an antigonadal effect and the weight reduction was maintained for 8 weeks, while the drug alone showed no significant effect.

Since the peracethylated cyclodextrins are ester-type derivatives, they are susceptible to alkaline hydrolysis resulting in re-formation of corresponding parent cyclodextrins and acetic acid in a 1:3 molar ratio in a fashion of the first-order kinetics. The peracethylated cyclodextrins are also degraded enzymatically with the rat skin homogenates. For example, the residual amounts of heptakis(2,3,6-tri-*O*-acetyl)-β-cyclodextrin and octakis(2,3,6-tri-*O*-acetyl)-γ-cyclodextrin were 72% and 60% after the 8 h of incubation, respectively, while heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin remained intact under the experimental conditions because of the ether-type derivative. Although there is no in-vivo kinetic evidence on the peracethylated cyclodextrins subcutaneously administered, once they are hydrolyzed into the respective parent cyclodextrins at the injection site, the resulting cyclodextrins are supposed to be easily absorbed and excreted into urine. Since the enzymatic hydrolysis of the peracethylated cyclodextrins at the injection site may proceed gradually as described above, it may be free from nephrotoxicity. In fact, no abnormality was found in the hemodiagnosis during the experiments through week 0 to week 4 after the subcutaneous administration of oily suspension containing the peracethylated cyclodextrins. Furthermore, it is likely that the peracethylated cyclodextrins, in addition to controlling the

release of buserelin acetate from the oil vehicle, may also act as stabilizers for the peptide against the enzymatic degradation at the site of administration. These facts suggest that the peracethylated cyclodextrins are preferable drug carriers for subcutaneous injection of peptides and proteins compared to heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin due to their possible bioabsorbable characteristics.

5. Sulfated Cyclodextrins as Heparinoids

The introduction of sulfate groups onto the hydroxyl groups of cyclodextrins confers biological activities, such as antiinflammatory and antilipemic activities, similar and sometimes superior to those of heparin on such derivatives.[9] Recently, cyclodextrin sulfates have been found to be effective in inhibiting cellular invasion by human immunodeficiency retrovirus[314−317] and to have antiangiogenic activity in combination with appropriate angiostatic steroids.[318,319] In our previous study a single intravenous administration of α-, β-, or γ-cyclodextrin sulfates at a dose of 1 g/kg was tolerated well in rats without conspicuous changes in blood chemistry values, while several parameters in rats receiving other polyanions including heparin, dextran sulfate, and poly(L-aspartic acid) at the same doses provoked renal or hepatic disorders.[78] In clinical practice, higher doses of heparinoids are sometimes associated with untoward reactions such as bleeding episodes due to their anticoagulant activity. The anticoagulant activity of cyclodextrin sulfates was about 100 times weaker than that of heparin on a weight basis and was comparable to that of dextran sulfate with a similar sulfur content. The effects of cyclodextrin sulfates on the cell membranes differ from those of the other hydrophilic cyclodextrins evaluated so far.[320−322] For instance, β-cyclodextrin sulfate showed a biphasic effect on the shape of erythrocytes, i.e. the crenation at relatively low concentrations and the invagination at higher concentrations. The β-cyclodextrin sulfate-induced membrane crenation arose from direct action on the membranes rather than cell metabolism-mediated effects. Unlike β-cyclodextrin, its sulfate was found to bind to the erythrocytes and may be confined to the outer surface of the membrane bilayer, which may expand the exterior layer relative to the cytoplasmic half, thereby inducing the cells to crenate. The lack of hemolytic activity of β-cyclodextrin sulfate may be due to the minimal capacity to solubilize the membrane lipids, together with a protective interaction with the membranes and an increase in osmotic pressure in the medium. Cyclodextrin sulfates would display their specific ability as a parenteral drug carrier over the cyclodextrins currently in use, in such a way that their weak heparin-mimicking activity could be utilized advantageously.

Basic fibroblast growth factor is a potent mitogen that stimulates the proliferation of a wide variety of cells and could play a crucial role in wound-healing processes. The therapeutic potential of basic fibroblast growth factor, however, has not been fully realized because of its susceptibility to proteolytic inactivation and short duration of retention at the site of action. Recent studies have demonstrated that sulfated oligosaccharides, including a sodium salt of β-cyclodextrin sulfate, have a high affinity for basic fibroblast growth factor and protect it from heat, acid, and proteolytic degradation. These sulfated oligosaccharides may bind close to the putative heparin binding domain, a cluster of several basic amino acid residues, on the surface of the basic fibroblast growth factor molecule, probably through an electrostatic interaction.[323,324] Unfortunately, the highly hydrophilic nature of the sodium salt of β-cyclodextrin sulfate is not suited to the design of basic fibroblast growth factor formulations with controlled-release features.

A water-insoluble aluminum salt of β-cyclodextrin sulfate was prepared, and its possible utility as a stabilizer and sustained-release carrier for basic fibroblast growth factor was evaluated.[325,326] An adsorbate of basic fibroblast growth factor with the aluminum salt of β-cyclodextrin sulfate was prepared by incubating the protein with a suspension of the aluminum salt of β-cyclodextrin sulfate in water. The mitogenic activity of basic fibroblast growth factor released from the adsorbate, as indicated by the proliferation of kidney cells of baby hamsters (BHK-21), was almost comparable with that of the intact protein. The aluminum salt of β-cyclodextrin sulfate significantly protected basic fibroblast growth factor from the proteolytic degradation by pepsin of α-chymotrypsin compared with their sodium salts and other oligosaccharides. The in vitro release of basic fibroblast growth factor from the adsorbate was sustained in proportion to a rise in the ratio of the aluminum salt of β-cyclodextrin sulfate to the protein. Of the basic fibroblast growth factor preparations tested, the adsorbate of basic fibroblast growth factor with the aluminum salt of β-cyclodextrin sulfate, when given subcutaneously to rats, showed the most prominent increase in the formation of granulation tissues, probably due to the stabilization and sustained delivery of the mitogen. These results suggest that the adsorbate of basic fibroblast growth factor with the aluminum salt of β-cyclodextrin sulfate has a potent therapeutic efficacy for wound healing and can be applicable to oral protein formulation for the treatment of intestinal mucosal erosions.

A hypothesis is proposed to provide a common mechanism for conventional antiulcer therapy, in which endogenous growth factors such as basic fibroblast growth factor play a central role.[327] An aluminum salt of sucrose sulfate (sucralfate) has a high affinity for basic fibroblast growth factor and protects it from acid degradation and inactivation. Oral administration of sucralfate elevates local levels of basic fibroblast growth factor in the ulcer bed, indicating that sucralfate acts as a potent angiogenesis stimulator, primarily on the basis of its ability to stabilize and slowly release locally available basic fibroblast growth factor. As described previously, the aluminum salt of β-cyclodextrin sulfate is a more potent stabilizer and sustained-release carrier for basic fibroblast growth factor than sucralfate.[326] The rigid macrocyclic structure of β-cyclodextrin sulfate imposes spatial constraints on the sulfate groups,

which enhances the charge density and the affinity for basic fibroblast growth factor. The oral administration of the aluminum salt of β-cyclodextrin sulfate tended to enhance the healing rate of acetic acid-induced gastric ulcers and cysteamine-induced duodenal ulcers, probably in a similar manner as sucralfate does. In particular, the oral administration of the aluminum salt of β-cyclodextrin sulfate loaded with basic fibroblast growth factor had the most prominent healing effects on the ulcers.[326]

In addition, the heparin-mimicking activity of cyclodextrin sulfates has been successfully applied to the inhibition of restenosis after the surgical approaches to the treatment of atherosclerosis,[328−330] the chromatographic separation of heparin binding proteins,[331,332] and the clinical diagnosis for direct determination of high-density and low-density lipoproteins in serum.[333,334]

C. Site-specific Delivery

Recently, intensive efforts have been made to design systems able to deliver drugs more efficiently to specific organs, tissues, and cells, etc. Cyclodextrin complexes are in equilibrium with guest and host molecules in water, the degree of the dissociation being dependent on the magnitude of the stability constant. This property of the complex is a desirable quality, because the complex dissociates to free cyclodextrin and drug at the absorption site, and only the drug in free form enters into systemic circulation. A typical example is the application of 2-hydroxypropyl-β-cyclodextrin to the chemical delivery system developed by Bodor,[335] which will be described below. On the other hand, 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 cyclodextrins. In this section, recent results on site-specific delivery using cyclodextrins are described, although there are presently very few reports from this area.

1. Chemical Delivery System

When a drug is covalently coupled to 1-methyl-1,4-dihydronicotinic acid through enzymatically labile linkage, its lipophilicity increases and allows selective delivery of drug molecules into the brain across the blood−brain barrier. After the entry into the brain, the dihydropyridine moiety is oxidized by oxidoreductase to 1-methylpyridinium 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 action of second 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 antagonist.[335] The problem, however, is that the prodrugs of the chemical delivery system are poorly water soluble due to the presence of the lipophilic moiety. 2-Hydroxypropyl-β-cyclodextrin 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 the estradiol/chemical delivery system (5 mg/kg) solubilized in 20% 2-hydroxypropyl-β-cyclodextrin 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 dimethyl sulfoxide.[17] It is apparent that 2-hydroxypropyl-β-cyclodextrin has the advantage over dimethyl sulfoxide from a safety viewpoint. The improvement of the chemical delivery system systems using 2-hydroxypropyl-β-cyclodextrin was reported for testosterone,[336] dexamethasone,[337,338] and benzyl penicillin.[339]

2. Brain Targeting

The specific delivery of potential neuropharmaceuticals to the brain is obstructed by the presence of 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.[340] 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 cyclodextrins to brain targeting are few. One of the examples is the β-cyclodextrin 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-cyclodextrin and in the latter conjugate all hydroxyl groups of cyclodextrins were further methylated to increase the lipophilicity.[341,342] 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 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 apply to other neuropharmaceuticals such as morphine. The β-cyclodextrin conjugate with *N*-leucine-enkephalin is of interest, because it has a vacant cavity and can include a neurotropic drug, dothiepine.[341] In general, the substituents introduced at primary hydroxyl groups of cyclodextrins through a spacer with appropriate lengths 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 a viewpoint of drug formulation, since two different drugs can be incorporated in the cyclodextrin molecule.

3. Colon Targeting

Colon targeting is essentially classified as a delayed release with fairly long lag time (cf. Figure 7), because the time required to reach the colon after oral administration is expected to be about 8 h in man.[343] When a cyclodextrin complex is orally ap-

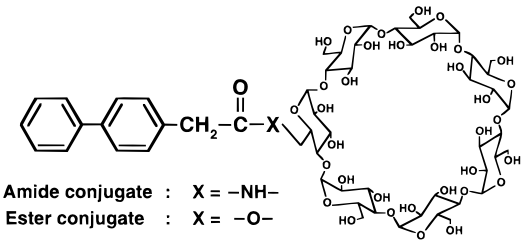


Figure 10. Structure of biphenylylacetic acid-β-cyclodextrin conjugate.

plied, it will readily dissociate in the gastrointestinal fluid, depending on the magnitude of the stability constant. This indicates that cyclodextrin complex is not suitable for colon-specific delivery, due to releasing the drug because of the dilution and/or competitive inclusion effects before it reaches to the colon. One of the advantage of the cyclodextrin−drug conjugate is that it can survive passage through stomach and small intestine, but the drug release will be triggered by enzymatic degradation of cyclodextrins in the colon.[63,344,345] Taking into account these factors, we have designed the amide and ester type conjugates of an antiinflammatory drug biphenylylacetic acid with three natural cyclodextrins, anticipating a new candidate for a colon targeting prodrug (Figure 10).[346,347]

Interestingly, the solubility of the cyclodextrin based prodrugs is related to the cavity size of cyclodextrins. For example, the extremely low solubility of the β-cyclodextrin conjugate was ascribed to the intermolecular association between the drug moiety and the neighboring β-cyclodextrin cavity. On the other hand, the highest solubility observed for the α-cyclodextrin conjugate may be due to the fact that α-cyclodextrin cavity is too small to accommodate the biphenylylacetic acid 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 biphenylylacetate, a simple ethyl ester of biphenylylacetic acid. Ethyl biphenylylacetate 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-cyclodextrin ester conjugates released biphenylylacetic acid 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, the α- and γ-cyclodextrin conjugates produced biphenylylacetic acid quantitatively, while the β-cyclodextrin conjugate released the drug in only small amounts, despite the significant disappearance of the β-cyclodextrin conjugate. Although the drug release patterns of the three cyclodextrin conjugates are different from each other, the in vitro data clearly suggest that the ester conjugates are first subject to the ring-opening of cyclodextrins by bacterial enzymes to give the triose and maltose conjugates through longer linear oligosaccharide conjugates. Thus, the ester linkage of the small saccharide−biphenylylacetic acid conjugates could be highly susceptible to hydrolysis. On the other hand,

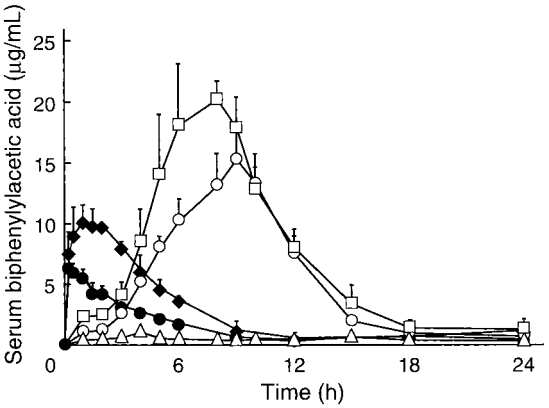


Figure 11. Serum levels of biphenylylacetic acid after oral administration of the biphenylylacetic acid ester conjugates with α-cyclodextrin (○), β-cyclodextrin (△), and γ-cyclodextrin (□), biphenylylacetic acid alone (●), or biphenylylacetic acid-β-cyclodextrin complex (◆) (equivalent to 10 mg/kg biphenylylacetic acid) to rats. Each point represents the mean ± standard errors of three experiments.

the amide conjugates hardly released the drug at all, despite fermentation to small saccharide−biphenylylacetic acid conjugates. Figure 11 shows the serum levels of biphenylylacetic acid after oral administration of three ester conjugates, compared with the drug alone and β-cyclodextrin complex in rats. As is expected, the fast dissolving form of β-cyclodextrin complex shows a rapid increase and decrease in the serum drug levels, compared with the drug alone. In the case of the β-cyclodextrin conjugate, little increase in serum level was observed, probably due to the slower drug release. However, the serum drug levels of the α- and γ-cyclodextrin conjugates increased after a lag time of about 3 h and reached maximum levels at about 9 and 8 h, respectively, accompanying a significant increase in the extent of bioavailability. The extents of bioavailability for the α- and γ-cyclodextrin conjugates were about 4 and 5 times larger than that for biphenylylacetic acid alone, respectively. In-vivo studies further revealed that biphenylylacetic acid 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.

On the basis of the above-mentioned results, the cyclodextrin-based colon-targeting prodrugs can be characterized as follows: In the case of ester type conjugates, drug release is triggered by the ring-opening of cyclodextrins, which consequently provides the 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 cyclodextrins. The amide linkage of the small saccharide−biphenylylacetic acid conjugates may be resistant to the bacterial enzymes and poorly absorbable from the intestinal tracts due to high hydrophilicity. Therefore, the ester type conjugate is preferable as a delayed release-type prodrug which can release a parent drug selectively in cecum and colon.

4. Cell Surface Targeting

β-Lactam antibiotics exert their lethal effect by inhibiting the synthesis of the bacterial peptidogly-

can, thereby disrupting the cell morphology and eventually resulting cell lysis and death.[348] Since the antibacterial effect of β-lactam is affected by the permeability of the outer membrane of bacteria, it is important for the evaluation of drug efficacy to measure the diffusion rate of drugs across the membrane. However, the β-lactamase enzyme expressed at cell surface interferes in the measurement. To solve this problem, a β-lactamase inhibitor which is water-soluble and could reach the cell membrane, but not penetrate it, must be prepared. Kurunaratne and co-workers chose β-cyclodextrin as a bulky moiety for prevention of drug entry of drug across channels in the bacterial outer membrane; thus, they prepared β-cyclodextrin conjugates linked to an antibiotic, methicilline, at the molecular terminus through spacers of different lengths (4.7−23.7 Å).[349] The extents of inhibition of surface β-lactamase of *P. Aeruginosa* in the presence of 10 mM conjugates were 6% using the conjugate with the spacer length 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 and co-workers reported that the α-cyclodextrin conjugate with antitumor sulfonylurea selectively blocks nicotinamide adenine dinucleotide oxidase activity at the external plasma membrane surface of the HeLa cell.[350]

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.[351] Therefore, cyclodextrin 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 cyclodextrin conjugates with mono- and di-saccharides such as glucose, galactose, mannose, fucose, etc., have been prepared and investigated for binding characteristics to sugar-specific receptors.[352−354] The β-cyclodextrin conjugates with galactose showed 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-cyclodextrin showed a high affinity (association constant 8730 M^{-1}) for the glucose-binding protein concanavalin A, a representative of a large family of lectins.[355] 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.[356] The sugar-substituted cyclodextrin derivatives may offer a new way of delivering drugs selectively to specific cell surface of organs such as liver and colon, although the uptake of drugs into cells may decrease due to the presence of a bulky, hydrophilic cyclodextrin moiety, as reported for the cyclodextrin conjugates with oligonucleotide and doxorubicin.[357,358]

V. Conclusions

The purpose of this review was to determine how well cyclodextrins satisfied the requirements for a drug carrier in drug delivery systems. Among the desirable properties of a drug carrier is that the carrier itself be bioadaptable particularly in parenteral applications; high quality and safe cyclodextrins such as 2-hydroxypropyl-β-cyclodextrin, 6-*O*-maltosyl-β-cyclodextrin, and sulfobutyl-β-cyclodextrin meet this standard better than other chemically modified cyclodextrins. The second desirable attribute for the drug carrier is the ability to control the rate and time of drug release. Multifunctional characteristics of peracylated cyclodextrins may serve as novel hydrophobic carriers to control the release of water-soluble drugs including peptide and protein drugs in various routes of administration. On the other hand, amphiphilic or ionizable cyclodextrins 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 valuable in the improvement of carrier properties of cyclodextrins. The final requirement of a drug carrier is its ability to deliver a drug to a targeted site; conjugates of a drug with cyclodextrins partially fulfill this requirement. As discussed in the previous section, conjugates of a drug with cyclodextrins can be a versatile means for the construction of colon-specific delivery system and will eventually form a new class of colon-targeting prodrugs.

In conclusion, cyclodextrins have significant potential as drug carriers in 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 cyclodextrin-based formulation.

VI. References

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