Pharmaceutical Applications of Cyclodextrins. 2. *In Vivo* Drug Delivery

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Abstract ☐ The objective of this Review is to summarize and critique recent findings and applications of both unmodified and modified cyclodextrins for *in vivo* drug delivery. This review focuses on the use of cyclodextrins for parenteral, oral, ophthalmic, and nasal drug delivery. Other routes including dermal, rectal, and pulmonary delivery are also briefly addressed. This Review primarily focuses on newer findings concerning cyclodextrin derivatives which are likely to receive regulatory acceptance due to improved aqueous solubility and safety profiles as compared to the unmodified cyclodextrins. Many of the applications reviewed involve the use of hydroxypropyl-β-cyclodextrins (HP-β-CDs) and sulfobutylether-β-cyclodextrins (SBE-β-CDs) which show promise of greater safety while maintaining the ability to form inclusion complexes. The advantages and limitations of HP-β-CD, SBE-β-CD, and other cyclodextrins are addressed.

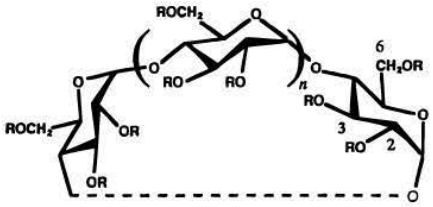
Introduction

The objective of this Review is to summarize and critique recent findings and applications of the use of unmodified and modified cyclodextrins for *in vivo* drug delivery. As part of this series, Loftssen and Brewster[1] reviewed the use of cyclodextrins for the solubilization, stabilization, and formulation of drugs through the formation of inclusion complexes while Uekama et al.[2] will summarize findings on the safety profile of cyclodextrins. Numerous other major reviews on the actual and potential pharmaceutical uses of cyclodextrins have been published.[3−11] Recently, as stated by Duchêne and Wouessidjewe,[3] the availability of new derivatives with better safety profiles has renewed interest in the *in vivo* uses of cyclodextrins. Also, as more products containing cyclodextrins move toward regulatory approval in the U.S. and elsewhere, the general acceptance by researchers and the pharmaceutical industry of specific cyclodextrins as enabling excipients is likely to increase. Although a number of products containing cyclodextrins have been approved for human use in Japan and Europe, no product has yet to be approved in the U.S. The approval of specific products by the Food and Drug Administration (FDA) in the U.S. will be of paramount importance to the commercial viability of cyclodextrins for worldwide pharmaceutical use. It is generally accepted that the lack of an FDA approved product, presumably due to actual or perceived safety concerns, continues to inhibit the universal acceptance of these valuable materials.

Cyclodextrin's Pharmaceutical Niche—In the early 1980s the high incidence of an anaphylactic reaction accompanying the parenteral administration of selected formulations led to questioning the use of surfactants which had been employed to solubilize or stabilize these formulations. This idiosyncratic histamine release was especially evident with the increased clinical use of the solubilizers Cremophor-EL present in cyclosporin A and taxol formulations, and Tween 80 which was used in an etoposide product. Other than the use of cosolvents, microemulsion dosage forms, pH adjustment for ionizable drugs, and experimental dosage forms such as microparticulates and liposomes, few new viable formulation options had been made available to address the problems associated with administering sparingly water soluble drugs in solution form. Although the use of cyclodextrins as potential solubilizing and stabilizing agents had been well recognized earlier, safety concerns limited their use for parenteral administration.

Cyclodextrins of pharmaceutical relevance are cyclic oligosaccharides composed of dextrose units joined through a 1−4 bond. Cyclodextrins with six to eight dextrose units have been named α-, β-, and γ-cyclodextrin, respectively (α-, β-, and γ-CD; Table 1). As discussed by Loftssen and Brewster,[1] cyclodextrins are capable of forming inclusion complexes with drugs. These noncovalent, inclusion complexes can have physical, chemical, and biological properties that are dramatically different from those of either the parent drug or cyclodextrin. These complexes can be used to increase solubility and dissolution rate, decrease volatility, alter release rates, modify local irritation, and increase the stability of drugs. The driving forces for inclusion complexation, including the re-

Table 1—General Structures[a] of Cyclodextrins Referred to in This Review and Their Abbreviated Names



Cyclodextrin	Abbrevia ion	R	*n*
α-Cyclodextrin	α-CD	H	4
β-Cyclodextrin	β-CD	H	5
γ-Cyclodextrin	γ-CD	H	6
Carboxymethyl-β-cyclodextrin	CM-β-CD	CH_2CO_2H or H	5
Carboxymethyl-ethyl-β-cyclodextrin	CME-β-CD	CH_2CO_2H, CH_2CH_3 or H	5
Diethyl-β-cyclodextrin	DE-β-CD	CH_2CH_3 or H	5
Dimethyl-β-cyclodextrin	DM-β-CD	CH_3 or H	5
Me hyl-β-cyclodextrin	M-β-CD	CH_3 or H	5
Random methyl-β-cyclodextrin	RM-β-CD	CH_3 or H	5
Glucosyl-β-cyclodextrin	G_1-β-CD	Glucosyl or H	5
Maltosyl-β-cyclodextrin	G_2-β-CD	Maltosyl or H	5
Hydroxyethyl-β-cyclodextrin	HE-β-CD	CH_2CH_2OH or H	5
Hydroxypropyl-β-cyclodextrin	HP-β-CD	$CH_2CHOHCH_3$ or H	5
Sulfobutyle her-β-cyclodextrin	SBE-β-CD	$(CH_2)_4SO_3Na$ or H	5

[a] Derivatives may have differing degrees of substitution of the 2, 3, and 6 positions.

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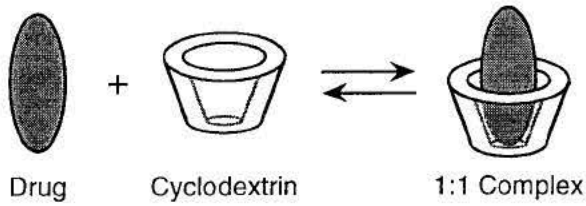


Scheme 1—Scheme illustrating equilibrium binding of drug and cyclodextrin to form a 1:1 complex.

quirement of the drug to "fit" into the cyclodextrin cavity, have been discussed elsewhere.[1,12–24]

Mechanisms of Drug Release from Inclusion Complexes—The rational design of formulations which take advantage of cyclodextrin inclusion complexation requires an understanding of the relationship between intrinsic drug solubility, the magnitude of the binding constant for the inclusion complex, and dilution effects. Most pharmaceutical agents form 1:1 complexes with cyclodextrins as described by Scheme 1. On the basis of the structure and properties of the drug as well as the cyclodextrin, higher order complexes are also possible.

The magnitude of the binding constant, $K_{1:1}$, defined by eq 1 for the interaction described by Scheme 1, is generally in the range 0–100 000 M^{-1}, with 0 being the value for a drug that is incapable of forming an inclusion complex and 100 000 M^{-1} being near the upper value observed experimentally for cyclodextrin complexes of drugs. In eq 1, $[\text{drug}]_{\text{complex}}$ represents the concentration of drug in the complex form, $[\text{drug}]_{\text{free}}$ represents the free drug concentration, and $[\text{cyclodextrin}]_{\text{free}}$ represents the concentration of free cyclodextrin.

$$K_{1:1} = \frac{[\text{drug}]_{\text{complex}}}{[\text{drug}]_{\text{free}}[\text{cyclodextrin}]_{\text{free}}} \quad (1)$$

For illustration of the relationship between drug solubility, magnitude of the binding constant, and dilution, a hypothetical drug (MW 400) with an intrinsic solubility of 10 $\mu g \cdot mL^{-1}$ (2.5×10^{-5} M) and a $K_{1:1}$ value of 10 000 M^{-1} for its interaction with a cyclodextrin of unlimited solubility will be examined. In the presence of the cyclodextrin, the solubility of the drug would be defined by eq 2. In eq 2, $[\text{drug}]_{\text{total}}$ represents the

$$[\text{drug}]_{\text{total}} = [\text{drug}]_{\text{intrinsic}} + \frac{K_{1:1}[\text{drug}]_{\text{intrinsic}}[\text{cyclodextrin}]_{\text{total}}}{K_{1:1}[\text{drug}]_{\text{intrinsic}} + 1} \quad (2)$$

total drug solubility, $[\text{drug}]_{\text{intrinsic}}$ represents the intrinsic solubility of the drug in the absence of the cyclodextrin, and $[\text{cyclodextrin}]_{\text{total}}$ represents the total molar concentration of cyclodextrin in the solution. In the presence of 0.1 M cyclodextrin, the solubility of the drug would be 8 $mg \cdot mL^{-1}$. An injectable dosage form containing 40 mg of this drug in 5 mL of 0.1 M cyclodextrin would contain only 50 μg of free or noncomplexed drug (0.125%), while the remaining 39.95 mg (99.875%) would be in the form of the inclusion complex. The ratio of free to complexed drug will change when this 5 mL formulation is injected intravenously by bolus dosing. For example, when this formulation is injected into a 70 kg subject with a plasma volume of about 3.5 L, the total drug and cyclodextrin concentrations would be 11.4 $\mu g \cdot mL^{-1}$ (2.85×10^{-5} M) and 0.143 mM, respectively. At these concentrations the percent free drug would be 43.9% with the balance still bound to the cyclodextrin. These values do not reflect drug and cyclodextrin binding to plasma proteins, uptake of drug into red blood cells or other tissues, and competitive displacement of the drug from the complex by plasma components. If

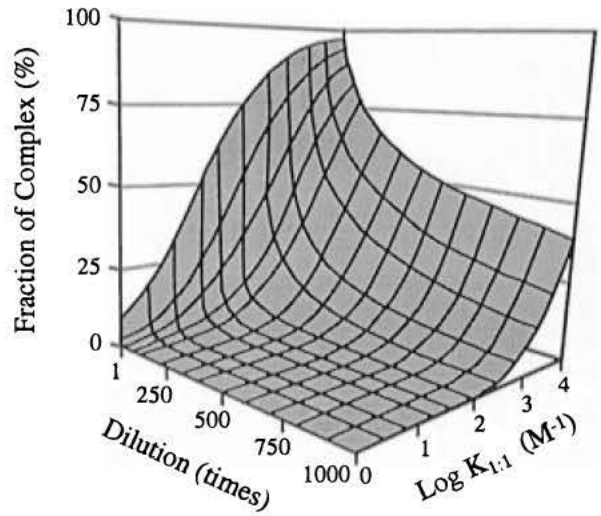


Figure 1—Relationship between fractional percent of a drug in its complex form as a function of the strength of the association constant, $K_{1:1}$, and dilution. The initial drug and cyclodextrin concentrations were set at 0.1 M, the range of $K_{1:1}$ values was 1–10 000 M^{-1}, and dilutions ranged over 1–1000 times. Reprinted with permission from ref 25. Copyright 1994 Harwood.

the drug has a more extensive volume of distribution (V_d), further dilution would occur and a smaller percentage would remain bound. Uekama et al.[25] have quantified the free and bound fractions of drugs to cyclodextrins as a function of drug concentration, binding constant, cyclodextrin concentration, and dilution. A graphical representation is shown in Figure 1. The kinetics of drug binding to cyclodextrins has been studied, and equilibrium binding is usually established with half-lives of much less than 1 s.[26–29] Therefore, the kinetics of dissociation is generally much faster than most physiological processes.

Frijlink et al.[30] studied the effect of dilution with plasma on hydroxypropyl-β-cyclodextrin (HP-β-CD) complexes of naproxen or flurbiprofen. They found experimentally that only small fractions of the drugs remained bound to the cyclodextrin in plasma *in vitro*. This effect was due not only to dilution but also to the competition between albumin binding of the two drugs and cyclodextrin binding. Also contributing to the low fraction bound was displacement of the drugs from cyclodextrin by a competing agent, plasma cholesterol.

The importance of changes in the ratio of free to complexed drug upon dilution of a sparingly water soluble drug in a cyclodextrin complex depends on the phase–solubility behavior of the system. If the cyclodextrin complex of a drug results from a 1:1 interaction, there is a linear increase in drug solubility with increasing cyclodextrin concentration (Figure 2A). Therefore, dilution of a true solution of the drug/cyclodextrin complex will not result in drug precipitation regardless of the extent of dilution. In the example discussed above where the 8 $mg \cdot mL^{-1}$ solution was diluted to 3.5 L, the final drug concentration was 11.4 $\mu g \cdot mL^{-1}$, of which the free drug concentration was 4.6 $\mu g \cdot mL^{-1}$ and the bound concentration was 6.8 $\mu g \cdot mL^{-1}$. The 4.6 $\mu g \cdot mL^{-1}$ free drug concentration is below the intrinsic solubility of the drug, set at 10 $\mu g \cdot mL^{-1}$, and precipitation of the drug will not occur.

Precipitation of the drug may occur on dilution, however, if there is a nonlinear relationship between drug solubility and cyclodextrin concentration. Figure 2B is a plot of solubility versus cyclodextrin concentration for the hypothetical drug described earlier where the solubility of 8 $mg \cdot mL^{-1}$ was achieved at 0.1 M cyclodextrin with a 1:1 interaction constant of 2000 M^{-1} (eq 1) and a 1:2 interaction constant, $K_{1:2}$, of 50 M^{-1} as described by Scheme 2.

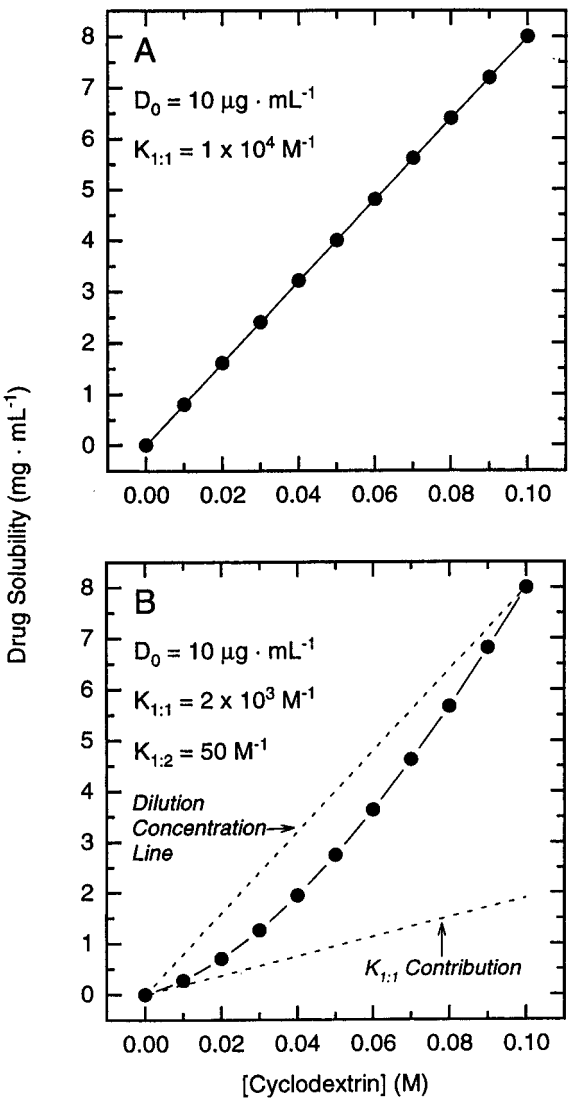
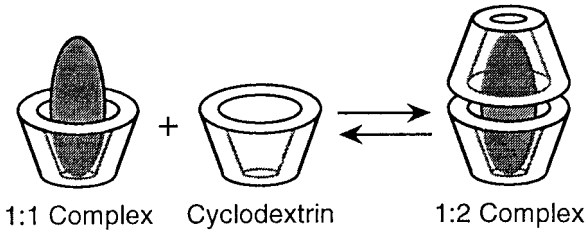


Figure 2—(A) Solubility versus cyclodextrin concentration for a hypothetical drug of MW 400 with an intrinsic water solubility of 10 μg·mL^{-1} (2.5×10^{-5} M) forming a 1:1 cyclodextrin complex with a drug with a $K_{1:1}$ association constant of 10 000 M^{-1}. (B) Solubility versus cyclodextrin concentration for a hypothetical drug of molecular weight 400 with an intrinsic water solubility of 10 μg·mL^{-1} (2.5×10^{-5} M) forming 1:1 and 1:2 cyclodextrin complexes with the drug wi h $K_{1:1}$ and $K_{1:2}$ constants of 2000 and 50 M^{-1}, respectively.



Scheme 2—Scheme illustrating equilibrium binding of a 1:1 complex of drug and cyclodextrin with a second molecule of cyclodextrin to form a 1 2 complex.

As can be seen from Figure 2B, there is a nonlinear increase in solubility with increasing cyclodextrin concentration. If we assume that an 8 mg·mL^{-1} solution of the drug in 0.1 M cyclodextrin is diluted 1:5 into a minibag, the final drug concentration would be 1.6 mg·mL^{-1} while the cyclodextrin concentration would be 0.02 M. According to the data in Figure 2B, the solubility of the drug in 0.02 M cyclodextrin is only 0.71 mg·mL^{-1}. Thus, precipitation of the drug on dilution may occur. Precipitation may occur in such systems at any dilution where the equilibrium drug solubility is lower than the dilution concentration line at a given cyclodextrin concentration. The problem of precipitation on dilution for those drugs which display the behavior just illustrated has recently been documented in the literature.[31] Theoretically, similar precipitation could also occur *in vivo* when such solutions are injected intravenously or by other parenteral routes.

Modified Cyclodextrins—Prior to the mid-1970s most pharmaceutical research on cyclodextrins focused on the unmodified α-, β-, and γ-CDs. The work of Frank et al.[32] and others[33] showing the nephrotoxicity of the unmodified cyclodextrins limited further studies on the parent cyclodextrins to those routes where systemic cyclodextrin absorption was limited, namely, nonparenteral routes.

Most drug molecules tend to interact more favorably with β-CD than α-CD because the 6 Å cavity diameter for β-CD accommodates aromatic groups found in many drug molecules. In contrast, the cavity diameter for α-CD tends to be too small for a favorable fit. Interactions can also be seen between many drugs and γ-CD. However, the cost of γ-CD has made its extensive use economically unfavorable. A severe limitation of β-CD is its limited water solubility of 18.6 mg·mL^{-1} or 16.4 mM. For example, a hypothetical drug with an aqueous solubility of 10 μg·mL^{-1} and a 1:1 binding constant with β-CD of 1×10^4 M^{-1} would have a maximum obtainable solubility of 1.3 mg·mL^{-1} in the presence of 16.4 mM β-CD. In addition, β-CD often forms B-type phase–solubility diagrams where the complexes themselves have limited aqueous solubility.[34]

Because of the solubility limits and the safety concerns with β-CD, numerous chemical modifications of the cyclodextrins have been made. Since the focus of this Review is on the *in vivo* applications of cyclodextrins, only those cyclodextrins which have been studied for their pharmaceutical utility will be discussed. The cyclodextrins of prime interest to pharmaceutical scientists consist of five general types. The first type consists of various methylated and alkylated cyclodextrins, especially 2,6-dimethyl-β-cyclodextrin (DM-β-CD) and randomly methylated β-cyclodextrins (RM-β-CD). The second type consists of the hydroxypropyl and hydroxyethyl cyclodextrins, especially 2-hydroxypropyl-β-cyclodextrins (HP-β-CD). Specific products include Encapsin and Molecusol. The third type consists of various branched cyclodextrins, especially glucosyl, diglucosyl (G_2-β-CD), maltosyl, and dimaltosyl cyclodextrins. The fourth type consists of carboxymethyl cyclodextrins, e.g., CM-β-CD, and associated derivatives. The fifth type consists of the sulfoalkylether cyclodextrins, especially sulfobutyl ethers (SBE-β-CD) of β-CD with degrees of substitution of 4 and 7, SBE4-β-CD and SBE7-β-CD, respectively. Specific products include Captisol, an SBE7-β-CD. The cyclodextrin derivatives discussed in this Review may be found in Table 1.

Pharmaceutical scientists have also investigated the antiangiogenic and antiviral properties of sulfated cyclodextrins; however, the ability of these derivatives to form inclusion complexes is rather minimal.[35–44] In addition, they have heparin-like activity resulting in an increase in blood clotting times which limits the cyclodextrin dose that can be administered to patients. Therefore, the use of sulfated cyclodextrins as potential complexing agents will not be discussed further. As noted for the sulfated cyclodextrins, safety is a primary concern when the advantages and disadvantages of the various cyclodextrins are considered. Uekama et al.[2] will address in detail the safety status of the various cyclodextrins in a Review to be published in a future issue. Questions on the safety of the various derivatives will be addressed briefly in this paper and then only as they affect the performance of the cyclodextrin.

Another very important factor in considering the pharmaceutical use of cyclodextrins is cost. Because cyclodextrins have molecular weights in the range of 1000−2000, 0.1 M solutions require 10−20% (w/v) cyclodextrin. Production of dosage forms on a commercial scale would quickly consume very large amounts of the cyclodextrin. Therefore the cyclodextrin must be reasonably inexpensive for the dosage form to be economically feasible. β-CD itself is quite inexpensive, and the costs of α- and γ-CDs are declining. Any modification of the cyclodextrin structure must entail relatively inexpensive reagents and purification procedures. However, well-characterized, pure, single-component materials are rare.

β-CD contains 21 hydroxyl groups, seven primary (the 6-hydroxy) and 14 secondary (the 2- and 3-hydroxyls). Methods have been developed with varying degrees of success either to derivatize all 21 of the hydroxyls or to selectively derivatize from one to seven of a particular hydroxyl group. Claims of selective derivatization are often overstated, and most claimed derivatives are mixtures. It may be very difficult to justify economically the development of a pure derivative unless it can be accomplished with a high yield, using simple and inexpensive reagents, and employing a purification procedure that can be easily scaled to produce metric ton quantities. The use of complex mixtures is not unknown to the pharmaceutical community. However, the methods used to characterize such mixtures need to be refined so that lot to lot reproducibility can be verified.

From the pharmaceutical viewpoint, not only must these complex mixtures be reproducible and well characterized lot to lot, but they must be free of all potentially reactive and toxic components. This is especially important in the case of unreacted parent cyclodextrin in products intended for parenteral administration. It is critical to have materials free of pyrogens and foreign proteins for cyclodextrins which are intended for parenteral use and to have the ability to sterilize the solution either by heat or filtration. These issues are not trivial and must be addressed before a particular cyclodextrin can be considered for pharmaceutical use.

Parenteral Applications of Cyclodextrins

Introduction—The major anticipated uses of cyclodextrins in parenteral drug delivery include solubilization of drugs, allowing for rapid and quantitative delivery of sparingly water soluble drugs for intravenous and intramuscular dosing, decreasing irritation at the site of administration of parenterally administered drugs, and stabilization of drugs unstable in an aqueous environment.

To date two types of parenteral studies have been published. In the first type a cyclodextrin has been used during animal testing to allow for a solution dosage form to be administered. Some of these studies have used cyclodextrins of questionable safety while other studies have not run controls which might have allowed the researcher to determine if the cyclodextrin altered the pharmacodynamics of the drug. In these cases the cyclodextrin was used as a tool and was not intended as a prototypical formulation for ultimate scaling to humans. In the second type of study the cyclodextrin was being evaluated as a potential tool for improved parenteral delivery of either a model drug or an actual clinical candidate. Nevertheless, both types of studies provide valuable insight into the potential uses of cyclodextrins as enabling excipients for parenteral drug delivery.

Aspects of the use of cyclodextrins to effect parenteral delivery of drugs have been reviewed previously.[3−11,25,45−60] The safety record of the cyclodextrin is perhaps more critical with parenteral delivery than with other routes of administration. Other routes such as oral may result in the systemic delivery of little or no cyclodextrin. Parenteral delivery, on the other hand, guarantees systemic delivery. Although the parenteral use of unmodified cyclodextrins and some modified cyclodextrins such as DM-β-CD has been attempted, their systemic safety records are unacceptable. Since the safety records of HP-β-CDs and SBE-β-CDs appear more promising, this section will focus primarily on their use.

The earlier Review in this series by Loftsson and Brewster[1] focused on the solubilization and stabilization of drugs. Although most of the examples in the current literature on *in vivo* applications have focused on the use of cyclodextrins to solubilize and/or to decrease the irritancy of drugs, future uses will also focus on their ability to stabilize drugs in aqueous solution, allowing for parenteral delivery. This is especially important for those unstable drugs for which long-term infusions might be desirable. For example, cyclodextrins are currently being studied extensively for their ability to stabilize a number of very unstable cytotoxic drugs.

Parenteral Delivery of Insoluble Drugs—Even though the safety of a particular cyclodextrin is the critical factor in its potential parenteral use, other factors also limit whether a cyclodextrin can be used to administer a given drug. One such factor is whether the target drug solubility can be achieved with the use of an acceptable cyclodextrin concentration. In addition, the cyclodextrin safety data must support the concentration and total dose of cyclodextrin required to solubilize the desired amount of drug. Other factors such as the linearity of the relationship between drug solubility and cyclodextrin concentration might also affect the acceptability of a given concentration or dose. If a nonlinear relationship exists and a very high concentration of cyclodextrin is needed to solubilize the drug, dilution of that sample either in a large volume parenteral (LVP) or on iv or im administration could lead to drug precipitation and complications. For example, the authors have noticed that some cyclodextrins could be used to solubilize the anticancer drug taxol (unpublished data). However, the relationships between taxol solubility and cyclodextrin concentration were such that any attempt to dilute the samples resulted in erratic precipitation of the taxol. Because nucleation either in an LVP or *in vivo* is a time dependent phenomenon, it might be possible to consider such metastable dosage forms even though precipitation can occur.[31]

Another critical factor would be whether the drug was completely released from the cyclodextrin dosage form. Drug release from the cyclodextrin dosage form can be determined by assessing the pharmacokinetics of the drug from the cyclodextrin solution as compared to other dosage forms. The pharmacokinetic studies would also indicate if the cyclodextrin altered the temporal pattern of the drug in plasma or blood, or the drug excretion profile. Either effect could lead to a change in pharmacodynamics and perhaps therapeutic efficacy and toxicity. Time profiles of the drug in various tissues would also be helpful in assessing the role of the dosage form. Few well-designed studies have addressed these latter issues to date.

Intravenous Administration—Most recent studies on the use of cyclodextrins to allow iv administration have utilized SBE4-β-CD or HP-β-CD since safety concerns with other cyclodextrins preclude their parenteral use. However, an early study by Shirakura et al.[61] involving β-CD is noted because the results demonstrated that iv β-CD significantly shortened the sleeping time induced by a series of barbituric acid derivatives. The amount of β-CD administered with the barbiturates, however, was about 0.244 $mg \cdot kg^{-1}$. This dose will show some significant renal toxicity. The possible mechanisms for the altered sleep times were speculated on by the authors, but none were conclusively proven. A similar study

which utilizes one of the safer cyclodextrins would provide valuable insight to the altered barbiturate activity.

Significant alteration in the pharmacokinetics of a drug was also demonstrated in the work of Frijlink et al.[30] This study evaluated the tissue distribution in rats of naproxen and flurbiprofen from HP-β-CD containing solutions as compared to solutions of the drugs dissolved in rat plasma. The tissue distribution in brain, liver, kidney, spleen, muscle, and plasma of naproxen 10 and 60 min after iv administration was unaffected by coadministration of 7% HP-β-CD. For flurbiprofen, however, at 10 min postdosing HP-β-CD produced significantly higher tissue levels in the brain, liver (most significant), kidney, and spleen but at 60 min postadministration only slightly higher, yet significant, levels in the brain tissue alone. Frijlink et al. speculated that the higher levels in some tissues reflected a transitory alteration in protein binding when HP-β-CD was used as the vehicle. In an *in vitro* study, HP-β-CD was able to compete with the protein binding for both naproxen and flurbiprofen but the effect was more pronounced with the flurbiprofen. Another control experiment where flurbiprofen was administered in a purely aqueous solution might have indicated whether administration in rat plasma also affected the relative results.

LaHann et al.[62,63] have studied the pharmacokinetics in dogs of *p*-boronophenylalanine following iv administration of 4.43 mg·kg^{-1} drug in an aqueous HP-β-CD solution (to effect greater solubility) versus a more dilute 0.96 mg·kg^{-1} aqueous solution. The pharmacokinetics of *p*-boronophenylalanine were found to be significantly different from the two dosage forms. The authors intimated that the pharmacokinetics of *p*-boronophenylalanine was dose dependent (nonlinear). Therefore, differences observed in the pharmacokinetics including longer half-life at the higher dose and a disproportionate increase in AUC with dose might be due to the dose differences. However, a direct effect of the HP-β-CD could not be ruled out. This issue could have been clarified by studying the pharmacokinetics of the drug at increasing doses in the presence of a fixed HP-β-CD dose level. Greater toxicity was seen with the HP-β-CD formulation, but this may have been due to the higher dose levels. Some toxicity from the HP-β-CD vehicle itself was noted in a few dogs. The etiology of this response was not assessed.

In those studies where control experiments could be performed, i.e., where the drug could be given by means other than the cyclodextrins, cyclodextrins have been found to not alter the intrinsic pharmacokinetics of the drug. For example, Arimori et al. showed that γ-CD did not alter the pharmacokinetics of thiopental in rabbits after iv administration.[64] The activity of the anesthetic propofol (2,6-diisopropylphenol) was no different when administered iv as a HP-β-CD solution versus a commercial oil in water emulsion.[65] The responses to increasing doses of the anesthetic isoflurane from a HP-β-CD solution were similar to those after inhalation of the agent.[66] Estes et al.[67] evaluated a nonsurfactant formulation that contained HP-β-CD for administration of alfaxalone (3α-hydroxy-5α-pregnane-11,20-dione, a steroid anesthetic) to rats and dogs. This formulation was compared to a veterinary product containing the surfactant Cremophor-EL. No difference in anesthetic activity was seen between the two formulations in rats. In dogs, the HP-β-CD formulation did not cause the significant histamine release and depressed respiratory rate and blood pressure drop seen with the surfactant formulation. More recently, the same research group provided additional data showing the usefulness of HP-β-CD in the parenteral administration of a series of other steroidal anesthetic agents.[68]

Doenicke et al.[69] compared the iv bolus pharmacokinetics and activity of the hypnotic agent etomidate (2 mg·mL^{-1}) from a 35% propylene glycol aqueous solution compared to a 3% HP-β-CD solution. The pharmacokinetics of etomidate were identical from the two solutions, and no differences in hypnotic effects were observed. A higher incidence of pain on injection was noted in patients receiving the propylene glycol solution (58% of the patients) as compared to patients receiving the HP-β-CD formulation (8% of the patients). Another study[70] did show a high incidence of pain (52%) from the HP-β-CD formulation when it is was assessed alone. Etomidate itself caused irritation and pain on injection.

Brewster et al.[71] were able to develop a parenteral formulation of the anticonvulsant drug carbamazepine through the use of HP-β-CD. Carbamazepine solubility increased nonlinearly with increasing HP-β-CD concentrations, suggesting complexes of higher order than 1:1 between carbamazepine and HP-β-CD. A tolerability and pharmacokinetic study was performed in epileptic patients by Löscher et al.[72] by comparing a 10 mg·mL^{-1} carbamazepine solution in 20% HP-β-CD to a 65% aqueous glycofural (PEG monotetrahydrofurfuryl ether) solution. The half-lives and AUC values for carbamazepine from HP-β-CD and glycofural were 0.603 $\pm$ 0.043 h versus 1.0 $\pm$ 0.014 h and 3.70 $\pm$ 0.72 μg·h·mL^{-1} versus 7.37 $\pm$ 0.72 μg·h·mL^{-1}, respectively. The longer half-life and higher AUC value (and therefore slower clearance) of carbamazepine and the delayed appearance of the epoxide metabolite of carbamazepine from the glycofural formulation strongly suggested that glycofural was an inhibitor of carbamazepine metabolism. Therefore, the nonidentical pharmacokinetics of carbamazepine from the HP-β-CD solution was most probably due to the negative effect of the glycofural in the control formulation rather than any catalytic effect of the HP-β-CD on carbamazepine elimination.

The pharmacokinetics of the steroids methylprednisolone, dexamethasone, and prednisolone have been evaluated from solutions of cyclodextrins compared to either cosolvent solutions or from water soluble prodrugs of the steroids. Arimori and Uekama[73] studied the iv pharmacokinetics of prednisolone in rabbits from β-CD and γ-CD aqueous solutions as compared to an aqueous solution of its phosphate ester prodrug. The cyclodextrin solutions and the solution of the prodrug showed identical pharmacokinetics. Dietzel et al.[74] compared the iv bolus pharmacokinetics in dogs of dexamethasone (5 mg·kg^{-1} from a 25 mg·mL^{-1} solution) from a 40% HP-β-CD solution compared to a molar equivalent of a water soluble dexamethasone phosphate prodrug. This work has been reviewed by Loftsson et al.[8]

For the phosphate ester prodrug to release dexamethasone it must be cleaved *in vivo* by phosphatase enzymes. The plasma concentration versus time curves for the dexamethasone from the two dosage forms were not identical. Plasma levels during the first hour were significantly higher from the HP-β-CD solution. However, the curves were superimposable after 1 h. There was no significant difference between the plasma AUC values ($\pm$SD) to infinity from the prodrug and the HP-β-CD formulation (5.48 $\pm$ 0.67 μg·h·mL^{-1} versus 6.67 $\pm$ 1.69 μg·h·mL^{-1}). The AUC values over the first hour were significantly lower from the prodrug formulation than from the HP-β-CD solution (1.04 $\pm$ 0.10 μg·h·mL^{-1} versus 1.63 $\pm$ 0.13 μg·h·mL^{-1}). The authors concluded that the lower early plasma concentrations may have been due to slow conversion of the prodrug to dexamethasone. Although the differences between the curves were not significant, there was a trend in the AUC values to infinity which suggested incomplete overall conversion of the phosphate ester prodrug to dexamethasone. This incomplete conversion may have been due to both slow and incomplete conversion of the prodrug. It is possible that metabolic or elimination pathways may have been competing for prodrug conversion to parent drug. Of additional interest was the higher renal clearance of dexamethasone with the HP-β-CD formulation, 0.91 $\pm$ 0.20 mL·min^{-1}·kg^{-1} versus 0.52

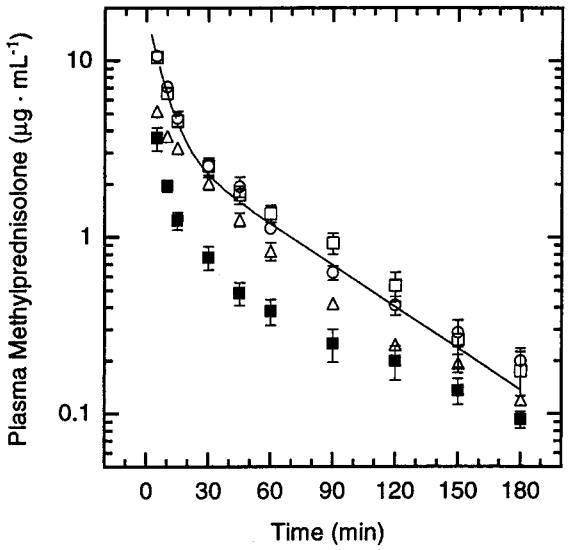


Figure 3—Plasma concentration (±SE) versus time profiles of me hylprednisolone after 20 mg·kg^{-1} iv dosing of methylprednisolone (or its molar equivalent) in anesthetized rats ($n = 6$). The solid line is the curve fit for the cosolvent data to a biexponential equation. Key: (□) cosolvent mixture, (○) 0.075 M SBE4-β-CD, (△) 21-phosphate ester, (■) 21-hemisuccinate ester. Reprinted with permission from ref 75. Copyright 1995 Elsevier.

± 0.20 mL·min^{-1}·kg^{-1} for the prodrug. The authors speculated that the higher clearance may have been due to decreased tubular resorption of the dexamethasone related to formation of an HP-β-CD complex of dexamethasone in the tubular fluid.

Stella et al.[75] compared the iv bolus pharmacokinetics in rats of methylprednisolone from an SBE4-β-CD solution (0.075 M), a cosolvent solution (60% PEG400, 12% ethanol, made up to 100% volume with water), and solutions of the 21-hemisuccinate and the 21-phosphate esters. The aim of the work was to investigate the effect of SBE4-β-CD on the pharmacokinetics of methylprednisolone as compared to the cosolvent mixture. Concurrent pharmacokinetic comparison of these formulations to solutions of the two prodrugs would provide insight into a formulation versus a chemical or prodrug approach to the parenteral delivery of a sparingly water soluble drug like methylprednisolone. Figure 3 shows the plasma concentration versus time profiles for the four formulations while Table 2 summarizes some of the relevant pharmacokinetic parameters determined from the data. There were no differences in any parameter when methylprednisolone from the SBE4-β-CD solution was compared to the cosolvent. There was, however, apparent incomplete release of methylprednisolone from the two prodrug formulations in the rats. On the basis of both their own data and various literature references, the authors concluded that the lower plasma concentrations and AUC values from the prodrugs were due to a probable sequential first-pass effect. Methylprednisolone is reversibly metabolized to methylprednisone in most animal species. Although the authors were not able to quantitate methylprednisone from all the animals because of an occasional analytical interference in its quantitation, the AUC values for methylprednisone were in the same rank order as those seen for the AUC values for methylprednisolone, i.e., SBE4-β-CD ≈ cosolvent > phosphate > succinate. These observations confirmed the formation of methylprednisone from methylprednisolone and the order of availability from the various dosage forms and prodrugs.

Although control experiments could not be performed, Uematsu et al.[76] studied the pharmacokinetics of a new prostaglandin I_2 analog in healthy volunteers from an α-CD product. The antifungal agent itraconazole was effective when administered iv in a 40% HP-β-CD solution.[77,78] HP-β-CD served to solubilize itraconazole. HP-β-CD was also effectively used to deliver systemically a sparingly water soluble, redox-based estradiol-chemical delivery system candidate.[79–82] Distelmans et al.[83] showed that HP-β-CD was effective in encapsulating the drug erbulozole to test its antitumoral and radioprotective potential, while Tuman et al.[84] found that a novel nonsteroidal aromatase inhibitor (R 76713) solubilized by HP-β-CD was effective in inhibiting the aromatization of exogenous rostenedione to estrone. The 10% HP-β-CD solution vehicle had no effect on this conversion. Many steroids interact very effectively with HP-β-CD and other cyclodextrins. The fact that the high dose of HP-β-CD did not affect the conversion supports the idea that cyclodextrins are unlikely to dramatically alter the distribution of endogenous and exogenous materials on dilution or in the presence of competing materials.

Järvinen et al.[85] were able to administer cinnarizine iv to dogs and to follow its pharmacokinetics. Cinnarizine solubility is very low, and Järvinen et al. were able to obtain sufficient solubility through a combination of pH adjustment and use of SBE4-β-CD. SBE4-β-CD was more effective than HP-β-CD in solubilizing cinnarizine. Determination of the pharmacokinetics of iv cinnarizine allowed the determination of the absolute bioavailability of cinnarizine from a series of potential oral dosage form strategies.

Thiazolobenzimidazole is a novel agent active *in vitro* against HIV-1. Its pharmacokinetics and tissue distribution as an HP-β-CD solution were tested in mice.[86] This formulation for thiazolobenzimidazole was initially developed by Tinwalla et al.[87] The same research group also produced interesting *in vitro* solubility data on the tripeptide HIV protease inhibitor kynostatin using a combination of ionization and complexation with HP-β-CD and SBE4-β-CD.[88] Torres-Labandeira et al. demonstrated the potential utility of HP-β-CD to solubilize the anticancer drug pancreastatin for iv administration.[31]

Hermens et al.[89] were able to assess the nasal bioavailability of 17β-estradiol by comparing serum levels to those following an iv dose in DM-β-CD solution to rats and rabbits. Few studies have utilized DM-β-CD as a parenteral drug carrier because of safety concerns, although some interest was expressed earlier in its use.[50]

Intramuscular, Intrathecal, Intracerebral, and Epidural Administration—Only a limited number of im studies have been published on the use of cyclodextrins. An early paper by Arimori and Uekama[73] assessed the pharmacokinetics of prednisolone in rabbits from both suspensions and solution forms containing β-CD and γ-CD complexes. In the suspension study, prednisolone serum levels from a prednisolone suspension and suspensions of the incompletely dissolved β-CD and γ-CD complexes were compared. The relative AUC values of prednisolone over 5 h from the three dosage forms were 1.45 ± 0.18 (±SEM), 1.79 ± 0.10, and 1.74 ± 0.14 μg·h·mL^{-1}, respectively. Although no statistical differences were noted in the AUC values, the C_{max} value from the prednisolone suspension was significantly lower when compared to the values from the complexes (0.61 ± 0.09, 1.26 ± 0.09, and 1.23 ± 0.11 μg·mL^{-1}). The greater solubility of prednisolone in the complexes led to higher release rates from the im site. The cyclodextrins had no effect on the relative C_{max} and AUC values when the water soluble prednisolone phosphate was administered im both in the absence and in the presence of β-CD and γ-CD. Of interest was the observation that the t_{max} values were significantly delayed in the presence of the cyclodextrins as compared to their absence.

Yoshida et al.[90] studied the pharmacokinetics of nimodipine in rabbits from im injections of a nimodipine suspension compared to a partially solubilized nimodipine suspension

Table 2—Average Methylprednisolone Pharmacokinetic Parameters (±SE) for Four Different Dosage Forms after Iv Administration (20 mg·kg^{-1}) to Anesthetized Rats ($n = 6$)[75]

Dosage Form	$t_{1/2}$ (min)	AUC(0–∞) (μg·min·mL^{-1})	Clearance (mL·min^{-1}·kg^{-1})	AUMC(0–∞) (μg·min^2·mL^{-1}) × 10^{-3}	MRT (min)	F(%)[a]
Cosolvent	41.5 ± 3.6	326.7 ± 20.6	62.7 ± 4.7	14.9 ± 2.5	42.0 ± 7.3	100
SBE4-β-CD	43.2 ± 2.0	317.4 ± 15.4	63.8 ± 3.1	13.2 ± 1.1	41.5 ± 2.1	97.1 ± 4.7
21-Phosphate	45.2 ± 4.3	199.2 ± 13.9[b]		9.9 ± 1.6	48.2 ± 4.0	59.2 ± 4.4[b]
21-Hemisuccinate	40.6 ± 13.5	108.4 ± 17.3[b]		7.0 ± 2.7[b]	55.5 ± 13.6	33.2 ± 5.3[b]

[a] Systemic availability. [b] Significantly different from cosolvent control ($p < 0.05$).

containing HP-β-CD. The relative AUC values up to 24 h postdosing were 31.90 ± 8.78 ng·h·mL^{-1} for nimodipine versus 82.55 ± 8.92 ng·h·mL^{-1} for the HP-β-CD formulation. HP-β-CD increased the apparent solubility of nimodipine in water. For example, the intrinsic solubility of nimodipine in water was 5.5×10^{-6} M, but in the presence of 0.05 M HP-β-CD the aqueous solubility increased to about 3×10^{-4} M. The nimodipine formulation containing HP-β-CD injected im was an amorphous material containing a 3-fold molar excess of HP-β-CD. On the basis of the phase–solubility diagram of Yoshida et al.,[90] only a small portion of the dose would be expected to be in solution if nonamorphous materials were used. The better performance of the HP-β-CD amorphous complex probably resulted from a much greater nimodipine concentration in solution than indicated from the intrinsic solubility measurements.

Poorly water soluble drugs given by the im route in various vehicles including cosolvent systems can precipitate at their injection site due to dilution of the cosolvents. Additionally, the cosolvents themselves can be irritating. In studies related to these issues, Stella et al.[91] recently published on the pharmacokinetics in rabbits of prednisolone administered im from an SBE4-β-CD solution (0.09 M, slightly hypertonic), a PEG400/alcohol/water mixture (4:1:5), and an isotonic aqueous solution of a phosphate ester prodrug of prednisolone. The study attempted to address a number of issues. The first was whether SBE4-β-CD itself caused tissue irritation on injection as measured by the release of the intracellular marker creatine kinase (CK). The SBE4-β-CD data was compared to that of the cosolvent mixture. The second issue was whether the presence of the steroidal anti-inflammatory prednisolone altered the tissue irritation. The third issue was whether the release of prednisolone was different from the SBE4-β-CD solution as compared to the cosolvent mixture or an isotonic solution of the phosphate ester. The data shown in Figure 4A,B addresses the first two issues. The SBE4-β-CD solutions showed no significant difference from normal saline (NS) in their ability to release CK from im injection while the cosolvent mixture did cause a very significant elevation of CK when compared to the controls. The slight elevation of CK levels from the SBE4-β-CD and NS was traced to the multiple venous punctures and not to the im injections. Prednisolone had no major effects on CK levels except to slightly decrease the apparent damage caused by the cosolvent mixture. Plasma concentration–time profiles for prednisolone from the cosolvent and SBE4-β-CD are shown in Figure 5. The differences between AUC values for the cosolvent as compared to the SBE4-β-CD solution were not statistically different; however, the C_{max} (±SE) from the SBE4-β-CD solution (6.45 ± 0.35 μg·mL^{-1}) was significantly higher than from the cosolvent (4.58 ± 0.59 μg·mL^{-1}). Deconvolution of the plasma concentration versus time data suggested possible precipitation of 20–30% of the prednisolone dose at the injection site from the cosolvent, which accounts for the longer apparent half-life of prednisolone from this vehicle (inset, Figure 5).

Some researchers have attempted to use cyclodextrins to alter the release rates of drugs from injection sites. Uekama et al.[92] and Matsubara et al.[93] studied the release after im

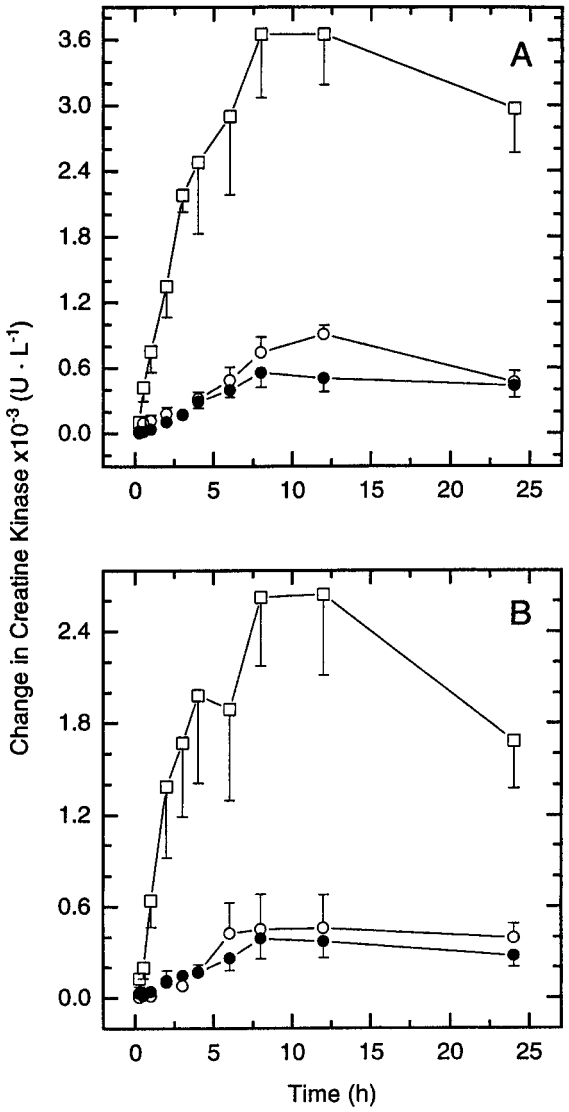


Figure 4—(A) Change in plasma CK levels (±SE) after im injection of 1 mL into both the right and left lumbar muscles of a PEG400/ethanol/water (40:10:50) mixture (□), a 0.09 M SBE4-β-CD solution (●), and normal saline (○) to rabbits ($n = 8$). (B) Change in plasma CK levels (±SE) after im injection of 1 mL into both the right and left lumbar muscles of prednisolone in a PEG400/ethanol/water (40:10:50) mixture (□), in a 0.09 M SBE4-β-CD solution (●), and from an isotonic prednisolone 21-phosphate solution (○) to rabbits ($n = 8$). Reprinted with permission from ref 91. Copyright 1995 Elsevier.

administration of the LHRH agonist buserelin acetate from oily suspensions in the presence of various cyclodextrins. The effects of HP-β-CD in altering the activity of a number of opioids and model drugs after intracerebral, intrathecal, and epidural administration have been mixed.[94–99] The results indicated that the effects of HP-β-CD varied with concentration. No dramatic long-term sustaining effect was observed in any of the studies although some interesting observations

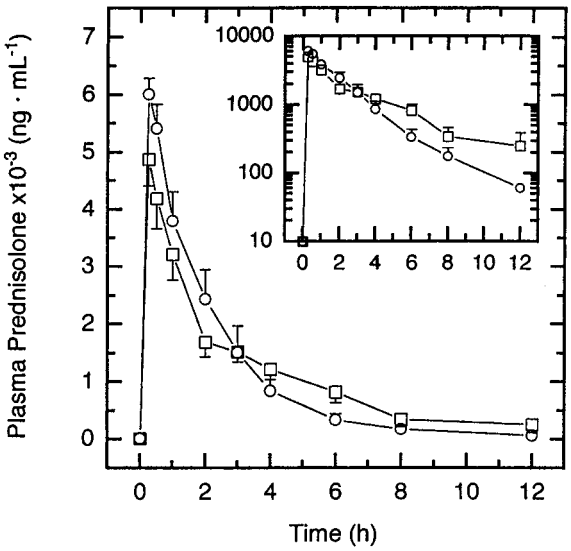


Figure 5—Concentration (±SE) versus time profiles for prednisolone after 5 mg·kg^{-1} im injections of a 1 mL solution into bo h the right and left lumbar muscles of rabbits ($n = 8$) from a cosolvent mixture (□) and from a 0 09 M SBE4-β-CD solution (○). Inset is the same data plotted on a semilog scale. Reprinted with permission from ref 91. Copyright 1995 Elsevier.

were made. Chatelut et al.[100] and Bonetti et al.[101] attempted to extend the release of methotrexate from liposomal preparations where HP-β-CD was used to keep the methotrexate inside the liposome.

Cyclodextrins and Site of Administration Irritation—Some cyclodextrins have been shown to be irritants while others have been found to be quite safe. Irritation can also come from the intrinsic toxicity of the drug itself or the vehicle in which the drug must be formulated. It is possible that drug- or vehicle-induced irritancy could be ameliorated by the formation of inclusion complexes.

In the last section, data on the im pharmacokinetics of prednisolone was presented along with data showing the lack of CK release from the injection site after administration of prednisolone as a SBE4-β-CD solution.[91] Numerous products exist that contain either surfactants and/or cosolvents that are irritating. Using cyclodextrins like HP-β-CD and SBE-β-CDs may allow for safer, less irritating formulations to be developed.

Only a limited number of studies have been performed to assess whether cyclodextrins can ameliorate the intrinsic irritancy of a drug after iv or im administration. There does appear to be a reasonable correlation between irritancy from an agent and the agent's ability to cause red blood cell hemolysis.[44,102−106] Even though β-CD is known to cause red blood cell hemolysis, Irie et al.[107] demonstrated that β-CD not only protected red blood cells from hemolysis caused by the neurological agent chlorpromazine hydrochloride but also decreased tissue damage to the *Musculus vastus lateralis* caused by im injection of chlorpromazine to rabbits. Chlorpromazine hydrochloride lowers the surface tension of aqueous solutions, and this effect is reversed in the presence of β-CD. After im injection, the C_{max} for chlorpromazine from the β-CD complex of chlorpromazine was significantly elevated at early time points compared to the C_{max} for chlorpromazine alone. The overall AUC values were also higher (127 ± 10 ng·h·mL^{-1} versus 114 ± 13 ng·h·mL^{-1}), but these differences were not significant. The authors suggested that the lower C_{max} values were due to chlorpromazine binding to tissues at the injection site or precipitation of drug at the injection site due to the poor solubility of chlorpromazine at physiological pH values. In an earlier study, Uekama et al. had demonstrated that β-CD did not affect the central nervous activity of chlorpromazine.[105] Svendsen[108] also demonstrated that β-CD could ameliorate the im irritancy of chlorpromazine but not oxytetracycline hydrochloride, a drug that did not interact with the β-CD.

Prochlorperazine, another neuroleptic, is capable of causing skin irritation. Uekama et al.[109] showed that both γ-CD and β-CD were able to lessen the skin irritancy of prochlorperazine while α-CD actually enhanced the irritancy. They concluded that the weak irritancy of γ-CD itself made it more usable than β-CD. Sato et al.[110] studied the effect of various cyclodextrins on red blood cell hemolysis caused by the drug tiamulin. γ-CD was most effective in preventing red blood cell hemolysis. The irritative effect of tiamulin, with and without γ-CD, was tested after *M. vastus lateralis* injection in rabbits. The irritation from the cyclodextrin complex was found to be less severe than that from the drug alone.[110]

Masuda et al.[111] studied the effect of the three unmodified cyclodextrins on the irritancy of flurbiprofen. On the basis of various determinations of binding constants, the interaction of flurbiprofen with the cyclodextrins was β- > γ- > α-CD. With respect to protection against red blood cell hemolysis, β-CD was greater than γ-CD while the β-CD complex with flurbiprofen provided the least severe irritation as compared to the free drug and the other complexes.

If irritancy of the cyclodextrin and its ability to ameliorate the local irritancy of agents is reflected by the hemolytic action of the cyclodextrins and alteration of hemolysis by materials like chlorpromazine, then it is likely that both HP-β-CD and various SBE-β-CDs will be much more useful than other cyclodextrins. This was confirmed for HP-β-CD by Yoshida et al.,[90] who showed that the irritation score of im nimodipine suspensions could be lowered from 1.88 ± 0.48 to 0.85 ± 0.44. Rajewski et al.,[112] Shiotani et al.,[44,113] and others have shown that the rank order hemolytic action of various cyclodextrins is DM-β-CD > β-CD > SBE1-β-CD ≈ HP-β-CD ≫ SBE4-β-CD > SBE7-β-CD. SBE-β-CDs were very effective in preventing the hemolytic action of chlorpromazine.[113] Well-designed studies should be undertaken to verify the protective action of HP-β-CD and SBE7-β-CD on the im, iv, and sc tissue irritancy of drugs known to be damaging at injection sites.

Other Studies—Parenteral administration of HP-β-CD has been proposed to lower serum cholesterol and vitamin A in a hypervitaminosis case. Using cyclodextrins to potentially remove agents such as cholesterol[54,57,114,115] and vitamin A[45,51,116,117] from the body have proven to be useful. These observations may imply that, since cyclodextrins are capable of removing unwanted agents from the body, they might also remove essential materials. The interaction of cyclodextrins with both cholesterol and vitamin A appears to require a 1:2 (drug to cyclodextrin) interaction. Therefore, only neutral cyclodextrins are likely to possess this ability since studies with charged cyclodextrins have suggested that 1:2 interactions are not favorable due to Coulombic repulsion.[118−125]

Cyclodextrins, other than those generally recognized as being potentially safe, have also been used to administer poorly water soluble drugs with the objective of being able to assess the activity of the agent.[118−120,122−130] Since these studies provide little information on the cyclodextrin per se, these papers are not extensively discussed here.

In summary, most if not all drugs are rapidly and quantitatively released from cyclodextrin solutions when these solutions are administered parenterally. Some cyclodextrins have been shown to perturb circulating levels of cholesterol and vitamin A in the case of a hypervitaminosis A case. Small increases in the urinary excretion of lipophilic drugs solubilized by cyclodextrins have been noted, but the extent does

not appear to perturb the overall kinetics. Those cyclodextrins that have acceptable safety profiles are likely to find extensive clinical use in solubilizing, stabilizing, and decreasing the irritancy of parenterally administered drugs.

Oral Applications of Cyclodextrins

Introduction—The major anticipated uses of cyclodextrins in oral drug delivery include increasing drug bioavailability through an increase in apparent rate and extent of drug dissolution, increasing drug stability or release time during gastrointestinal (GI) transit through modification of drug release site and/or time profile, decreasing local tissue irritation, and masking of objectionable taste.

As in the parenteral applications of cyclodextrins, not only has this class of molecules found use in the formulation of potential drug products but they have also been used in animal drug testing to allow for solution dosage forms to be administered.[127,131] The use of cyclodextrins to increase the oral bioavailability of a drug through the increase of apparent drug dissolution rate has usually involved the use of the parent cyclodextrins or their more water soluble derivatives. Such applications have been successful when the rate-limiting step in drug absorption is dissolution of the drug itself and not absorption across the GI mucosa.

While many of these studies do not correlate the increased bioavailability with increased dissolution rate, most reports include the administration of uncomplexed drug or a commercial formulation to demonstrate the relative bioavailability. It is important to note that few studies have investigated whether apparent cyclodextrin dependent increases in oral bioavailability are solely a result of increasing the rate at which drug is available for absorption or whether cyclodextrin-induced changes in mucosal permeability play a supporting role. In contrast to the use of hydrophilic cyclodextrins to increase oral bioavailability, the use of cyclodextrins to extend the release of drugs during GI transit usually has involved the use of more hydrophobic cyclodextrin derivatives while studies aimed at alleviating unwanted effects (drug stability, variable absorption of weak bases, etc.) resulting from the low pH of the stomach have employed cyclodextrin derivatives which exhibit pH dependent solubility behavior. All these studies provide valuable insight into the potential uses of cyclodextrins in oral drug delivery.

Summary of Reviews to Date—Uekama et al. have recently published a general review[25] on applications of cyclodextrins including a brief overview of the GI absorption of the cyclodextrins themselves. As noted by Uekama et al., Irie et al.[132] demonstrated that small amounts of β- and γ-CD were absorbed by intestinal segments under bile duct ligation during *in situ* loop perfusion studies in rats. Therefore, the possibility of intestinal absorption of the cyclodextrins should not be totally disregarded. Others have also observed the absorption of small amounts of cyclodextrin through the GI tract of rats when given alone[133,134] or as a complex with a drug.[135,136] Uekama et al.[25] also briefly reviewed the absorption-enhancing effects of cyclodextrins given by various routes including oral administration. While it is correct to assume that the largest impact of cyclodextrins on oral drug absorption results from an increased rate of drug dissolution of the complexed drug, the magnitude of the stability constant between the drug and the cyclodextrin should also be considered since only the free, uncomplexed drug is absorbed through the GI mucosa. However, as noted by Uekama et al.,[25] it is possible that cyclodextrins may affect drug absorption through modification of the mucosal membrane. Free cyclodextrin may remove membrane components,[132,137] thereby modifying the transport properties of the membrane and facilitating absorption of the drug. Such properties would not necessarily be hindered by derivatization of the cyclodextrin.[138]

Szejtli reviewed the physical and chemical mechanisms by which cyclodextrins increase dissolution rate and bioavailability.[60] The reader is directed to this publication for a complete discussion of this topic. Szejtli also has a very good discussion and theoretical simulation of the effect of the complexation stability constant and the ratio of drug to cyclodextrin on the blood level of the drug. According to the model employed by the authors, at drug to cyclodextrin ratios of unity an increase in peak blood levels and bioavailability over that of a cyclodextrin-free formulation would be predicted over a range of reasonable stability constants. At high stability constants and in the presence of molar excesses of cyclodextrin, however, the model suggests that the peak blood levels and bioavailability attained may actually be lower for a formulation containing cyclodextrin relative to one containing only drug. This effect would be exaggerated if the drug had a narrow absorption window. It should be noted that the model does not include competition for the cyclodextrin cavity by other molecules present in the GI tract.

Szejtli also briefly discusses the limitations of oral dosage forms containing cyclodextrins with regard to total dosage unit mass and drug content. The amount of drug which can be delivered in a unit dose is limited due to the differences in molecular weight of the drug and cyclodextrins (approximately an order of magnitude in most cases) and less than mole per mole binding of drug and cyclodextrin. This effect is important especially since many of the hydrophilic cyclodextrin derivative drug complexes cannot readily or economically be isolated as the pure complex. In many cases, therefore, a 500 mg tablet may only contain 5–25 mg of active drug, assuming that no other excipients in addition to the cyclodextrin are present. Both of the referenced reviews have very complete citations of the major publications concerning oral applications of cyclodextrins.

Increased Oral Bioavailability Resulting from Cyclodextrin Complexation—As mentioned previously, observed increases in the oral bioavailability of a drug from a cyclodextrin-containing formulation predominantly result from increases in the apparent solubility of the drug. Many recent studies have used more hydrophilic derivatives of β-CD in their investigations. Recently, Järvinen et al. reported on the increased oral bioavailability in beagle dogs of the weak base cinnarizine from formulations containing HP-β-CD and SBE4-β-CD.[85] These studies consisted of two parts. In the first study, 12.5 mg of cinnarizine in 10 mL of SBE4-β-CD solution was administered iv as a control. Additionally, three oral formulations consisting of 25 mg of cinnarizine in solution in either 75 mM SBE4-β-CD or HP-β-CD and as a suspension in phosphate buffer were given to fasted dogs via a gastric tube to complete a 4×4 crossover study. In the second study, 25 mg of cinnarizine was given in a capsule to fasted dogs via a gastric tube in the absence and presence of SBE4-β-CD. Blood samples were drawn for approximately 24 h postdosing, and cinnarizine levels were determined with a spectrofluorometric HPLC method.

The authors found that the iv pharmacokinetics followed triexponential behavior with a terminal half-life of approximately 13 h. The cinnarizine suspension and capsule gave absolute bioavailabilities of $8 \pm 4\%$ and $0.8 \pm 0.4\%$, respectively, with wide interanimal variation. The complexed cinnarizine in the capsule gave a bioavailability of $38 \pm 12\%$ while the solutions with SBE4-β-CD or HP-β-CD gave values of $60 \pm 13\%$ and $55 \pm 11\%$, respectively (Figure 6). The authors argued that the solutions demonstrated excellent bioavailability because cinnarizine is well absorbed when presented in solution and that the SBE4-β-CD containing

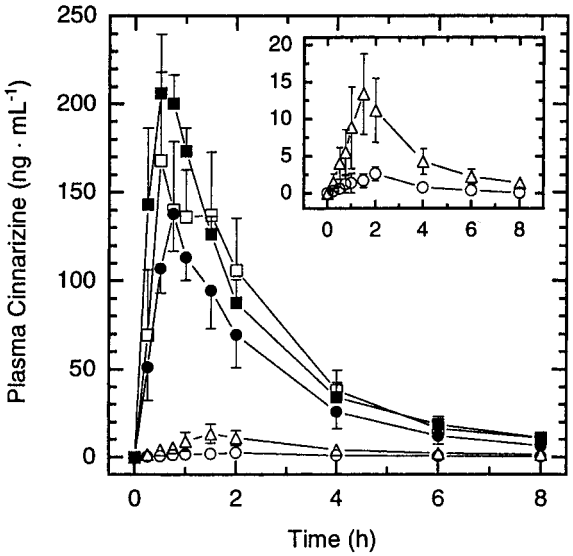


Figure 6—Cinnarizine plasma concentration in dogs (mean ± SE, $n = 4$) after oral administration of 25 mg of cinnarizine. Key: (■) HP-β-CD solution, (□) SBE4-β-CD solution, (●) capsule containing solid SBE4-β-CD complex of cinnarizine, (△) cinnarizine aqueous suspension (pH 4.5), (○) capsule containing cinnarizine but no SBE4-β-CD. Reprinted with permission from ref 85. Copyright 1995 American Chemical Society.

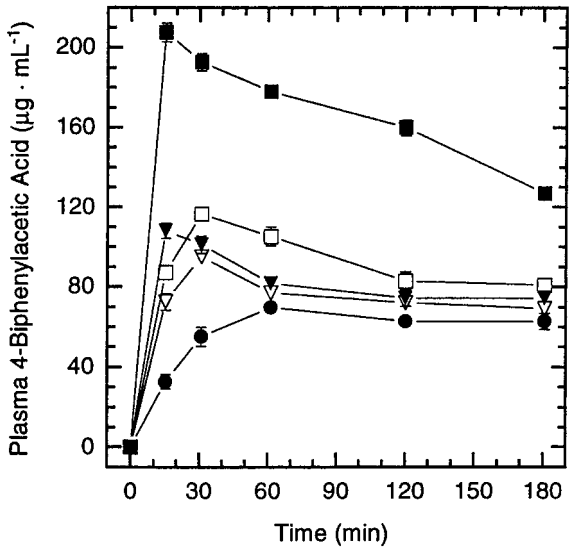


Figure 7—4-Biphenylacetic acid (BPAA) plasma levels in rats following oral administration of 10 mg·kg^{-1} BPAA. Values are the mean ± SE of three HPLC determinations. Key: (●) BPAA, (▽) BPAA and β-CD, (▼) BPAA and HP-β-CD, (□) BPAA and TM-β-CD, (■) BPAA and DM-β-CD. Reprinted with permission from ref 148. Copyright 1995 Royal Pharmaceutical Society of Great Britain.

capsule demonstrated elevated bioavailability because of rapid dissolution and release of cinnarizine.

Similar recent studies wherein the bioavailability or efficacy of drug was improved include the use of β-CD to improve the antiiflammatory activity in rats of piroxicam[139] and hamycin.[140] Recently, HP-β-CD was shown to double the bioavailability in rats of carbamazepine[141] and increase carbamazepine absorption in dogs 5.6 times over that of the commercial Tegretol formulation.[142] In other studies, HP-β-CD was shown to double the levels of ellagic acid, an antimutagenic and anticarcinogenic natural product analog, in the lungs of mice following oral administration.[143] HP-β-CD was also shown to improve the efficacy of itraconazole in mice[144] and rabbits.[145] The *in vivo* applications of other cyclodextrin derivatives have also been published recently. Fujitani and Wakitani[127] used α-CD to help administer OP-41483, a PGI_2 analog, to guinea pigs.

As previously discussed, these examples demonstrating increased bioavailability involve drugs which are hydrophobic and prone to dissolution rate limited absorption. This point should not be overlooked by the formulator when considering the incorporation of cyclodextrin into a dosage form in an attempt to increase bioavailability. For example, Cundy et al. recently reported on the use of HP-β-CD for formulation of a bis[(pivaloyloxy)methyl] (bis(POM)) prodrug of 9-[2-(phosphonylmethoxy)ethyl]adenine (PMEA) for bioavailability studies in monkeys.[146] The intestinal permeability of PMEA is limited due to the dianionic phosphonate group, and the bis(POM) derivative was theorized to be more lipophilic and, therefore, better absorbed. The authors found, however, that the bioavailability of the HP-β-CD complex as measured by PMEA in the systemic circulation was no better than that of an aqueous suspension of bis(POM)−PMEA or a cosolvent solution in PEG400. The observed equivalence suggests that dissolution is not the rate-limiting step in absorption of this prodrug. In fact, reversion of the prodrug to anionic PMEA in the GI tract or equivalent PMEA absorption from each of the formulations with transepithelial or first-pass reversion to the parent drug were suggested as explanations of the observed equivalence.

Mucosal permeation enhancement of the drug effected by cyclodextrins may also play a role in increased drug absorption. Shao et al.[147] recently reported on the use of DM-β-CD to increase the bioavailability of insulin in rats. In these studies, the relative effectiveness of DM-β-CD and HP-β-CD in enhancing the enteral absorption of insulin was evaluated in the lower jejunal and upper ileal segments of the rat using an *in situ* closed-loop method. The combination of 10% (w/v) DM-β-CD with 0.5 mg·mL^{-1} insulin in a PBS solution produced a bioavailability of approximately 5.6% compared to 0.06% for a PBS solution. Interestingly, an insulin and PBS solution containing 10% (w/v) HP-β-CD did not increase the bioavailability of insulin in this model. The authors argue that DM-β-CD must fluidize the mucosal membrane thereby enhancing insulin uptake since light microscopic inspection of the tissue indicated no observable damage.

The effect of cyclodextrin derivative type on bioavailability and permeability enhancement was also investigated by Puglisi et al.[148] In these studies the gastric tolerability, absorption, and pharmacological activity of 4-biphenylacetic acid (BPAA), a nonsteroidal anti-inflammatory drug, were investigated in the rat following oral administration of complexes with β-CD, DM-β-CD, 2,3,6-tri-*O*-methyl-β-CD (TM-β-CD), and HP-β-CD. It should be noted that the BPAA and the β-CD and TM-β-CD complexes of BPAA were administered as suspensions. As depicted in Figure 7, the authors found that the bioavailability of BPAA was increased by complexation with all these derivatives with AUC values in the following order: DM-β-CD (30 609 h·μg·mL^{-1}) > TM-β-CD (16 210 h·μg·mL^{-1}) > HP-β-CD (14 444 h·μg·mL^{-1}) > β-CD (13 206 h·μg·mL^{-1}) > BPAA alone (10 736 h·μg·mL^{-1}). The AUC values also paralleled results from the carrageenan paw edema test.

With regard to gastric tolerability, BPAA complexed with DM-β-CD, HP-β-CD, or β-CD exhibited better tolerability relative to the uncomplexed drug while the TM-β-CD complex produced gastric lesions similar in extent to those produced by BPAA alone. Both TM-β-CD (500 mg·kg^{-1}) and DM-β-CD (1000 mg·kg^{-1}) produced gastric lesions at 21 h postadministration when these cyclodextrin derivatives were administered orally while no lesions were found when β-CD and HP-β-CD were administered under these conditions. The authors maintain that absorption of BPAA is primarily influenced by the stability constant of the complex along with interactions of the cyclodextrin derivatives with the gastric mucosa. The

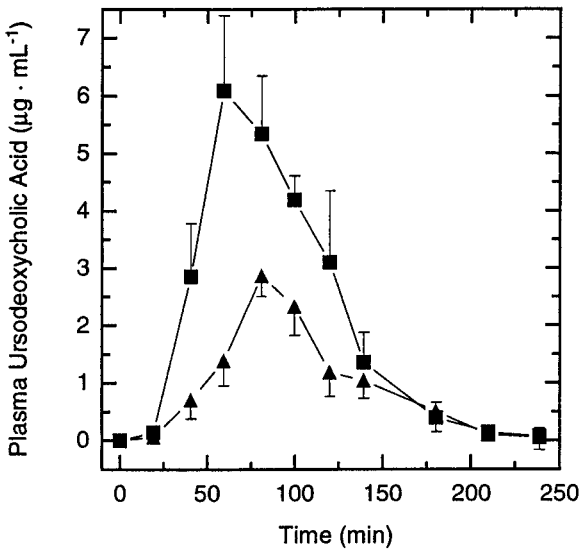


Figure 8—Ursodeoxycholic acid (UDCA) plasma concentrations in six healthy volunteers following oral administration of tablets containing 425 mg of UDCA in the inclusion complex of HP-β-CD (■) or of commercial tablets containing 450 mg of free UDCA (▲). Bars represent SD. Reprinted with permission from ref 153. Copyright 1995 Academic.

rate of dissolution was ruled out as playing a role in the absorption process on the basis of earlier work from this group[149] which showed that cyclodextrin complexes of BPAA are rapidly dissolved in water and do not form precipitates in acidic environments as low as pH 2. In support of their hypothesis concerning the role of permeation enhancement by cyclodextrins, the authors cite previously published work showing that DM-β-CD can enhance the nasal absorption of insulin[150] and can improve cell permeability by solubilizing specific plasma membrane lipid components[48,151] including those of the GI mucosa.[152] While it is difficult to ascertain from these studies the contribution of the permeability enhancement by cyclodextrin to overall absorption, the formulator should be aware that the potential does exist with certain cyclodextrin derivatives to alter membrane permeability. While it is of obvious importance in acute cases such as the exacerbation of GI irritation by drugs like the nonsteroidal anti-inflammatories, the chronic oral use of certain cyclodextrin derivatives known to interact and bind membrane components warrants consideration.

As in the previous examples, animal models have historically been employed to investigate the effect of cyclodextrin on bioavailability. As several cyclodextrin derivatives near regulatory approval, reports on human bioavailability studies using this technology continue to increase. For example, Panini et al. reported on the improvement of ursodeoxycholic acid (UDCA) bioavailability in healthy human volunteers through HP-β-CD complexation.[153] Oral UDCA treatment is useful for the dissolution of cholesterol gallstones and is beneficial in the treatment of biliary cirrhosis. It is available commercially as Ursacol tablets. The authors prepared tablets of a HP-β-CD complex of UDCA which contained microcrystalline cellulose and starch. These tablets had disintegration times identical to those of the commercial Ursacol tablets. The dissolution profiles of the two formulations were dramatically different, however, with dissolution of the commercial tablet being much slower and incomplete. The AUC and C_{max} values of the HP-β-CD complex were more than twice those of the commercial tablets (Figure 8) with the time of maximum concentration being 50% sooner with the complexed drug as compared to the commercial tablet. The authors attribute the improved performance of the cyclodextrin-containing formulation to the increased dissolution rate.

Other applications of HP-β-CD in human studies include a report by Hoon et al.[154] on the bioequivalence in postmenopausal women of estradiol formulations containing HP-β-CD given sublingually and orally versus micoronized estradiol by these routes. The authors found that the sublingual route produced significantly higher peak estradiol concentrations than the other routes; however, all formulations produced similar LH suppression over 24 h. Stuenkel et al.[155] also used HP-β-CD for sublingual administration of testosterone in hypogonadal men. The subjects received either a 2.5 or 5.0 mg tablet of the HP-β-CD complex of testosterone three times daily for 7 days. Although no comparison to a formulation without HP-β-CD was made, the authors found that the cyclodextrin complex provided rapid adsorption of testosterone following sublingual absorption and produced systemic testosterone levels which were 63 times higher than baseline. Pitha et al.[156] also used HP-β-CD to study the sublingual absorption of progesterone, testosterone, and estradiol and found them to be systemically available presumably as a result of minimizing first-pass metabolism.

β-CD has also been used in formulations for oral administration. While these reports will not be extensively reviewed here, the subject of each publication will be addressed for the reader's information. Ricevuti et al.[157] investigated the pharmacokinetics in healthy volunteers of dipyridamole and its β-CD complex following single and multiple dosing and found better bioavailability from the complex. Yusuff et al.[158] observed improved spironolactone bioavailability from a β-CD complex and a micronized powder versus the commercial product, Aldactone. Otero-Espinar et al.[159] found no difference between the bioavailability in healthy volunteers of a β-CD complex of naproxen and that of naproxen and freeze-dried naproxen while the dissolution of the complex at pH 1.2 was considerably faster. The authors suggest that the lack of correlation between the dissolution data and the *in vivo* data is a result of a short and variable residence time of the formulation in the stomach followed by fast dissolution in the small intestine. Hildebrand et al.[160] employed a β-CD complex of cicaprost to further a dose dependence study in male volunteers. The dose titration study confirmed the pharmacological potency and oral availability of the drug. The use of β-CD in an *in vitro* and *in vivo* evaluation of spray-dried diazepam formulations was investigated by Bootsma et al.[161] The authors correlated increased *in vivo* absorption of cyclodextrin-containing formulations with *in vitro* dissolution differences which were only observed under low agitation intensities, potentially analogous to the *in vivo* situation. In single-dose and multiple-dose steady-state studies, Deroubaix et al.[162] studied the pharmacokinetics and oral bioavailability in healthy human volunteers of a β-CD complex of piroxicam. The authors found that the bioavailabilities of the β-CD complex and uncomplexed drug were equivalent; however, the absorption rate was faster from the β-CD dosage form. On repeated dosing the cyclodextrin dosage form yielded the same steady-state systemic concentrations of piroxicam as a commercial capsule.

Modification of Drug Release Site and/or Time Profile—As mentioned previously, the use of cyclodextrins to extend the release of drugs during GI transit usually has involved the use of more hydrophobic cyclodextrin derivatives such as the peracylated series of β-CD. In addition, the hydrophilic cyclodextrin derivatives have been joined with other delayed-release technologies to produce dosage forms with an immediate release (cyclodextrin driven) and a delayed release component.

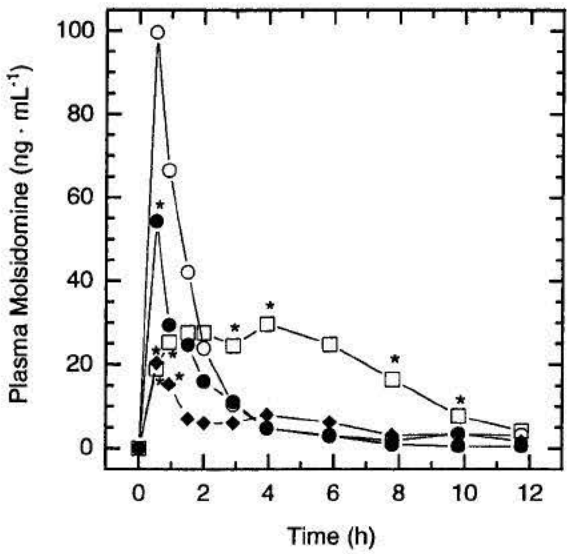


Figure 9—Plasma levels of molsidomine after oral administration of capsules containing molsidomine or its peracylated β-CD complexes (equivalent to 10 mg of molsidomine) in dogs. Key: (○) molsidomine alone (starch diluent), (●) peracetyl-β-CD complex, (□) perbutanoyl-β-CD complex, (◆) perhexanoyl-β-CD complex. Each value represents the mean of 3–6 dogs. (*) $p < 0.05$ compared to molsidomine alone. Reprinted with permission from ref 163. Copyright 1994 Royal Pharmaceutical Society of Great Britain.

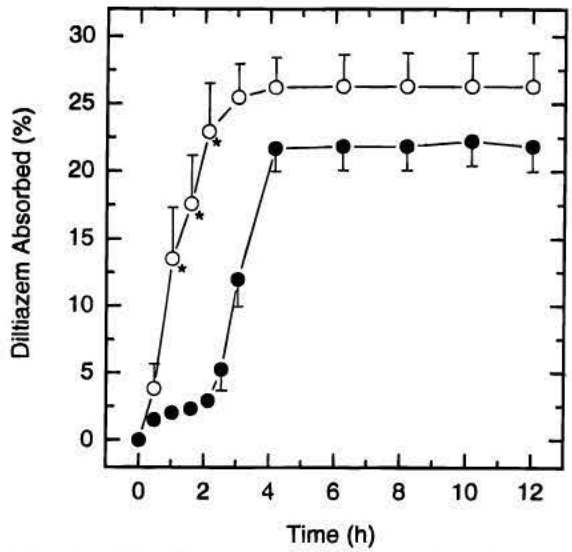


Figure 10—Absorptivity curves generated by deconvolution of diltiazem plasma level–time curves. Key: (○) high gastric acidity, (●) low gastric acidity. (*) $p < 0.05$ compared to high gastric acidity dogs. Reprinted with permission from ref 166. Copyright 1993 Elsevier.

For example, Uekama et al.[163] investigated the use of peracylated β-CD derivatives with alkyl chains from acetyl through octanoyl for modifying the release and absorption in dogs of the peripheral vasodilator molsidomine. Molsidomine was chosen as a model drug because it has a short biological half-life in humans (2.1−2.7 h) and an acceptable aqueous solubility (2.5 mg·mL^{-1} at 25 °C). The authors found that, with increasing alkyl chain length, the melting points and solubilities in 80% (v/v) ethanol/water decreased. *In vitro* drug release rates were retarded by complexation with the peracylated derivatives and decreased with the decreasing order in the solubility of the derivatives. The peracetyl-, perbutanoyl-, and perhexanoyl-β-CDs were chosen for *in vivo* oral absorption studies. The molsidomine complexes in gelatin capsules were administered to fasted beagle dogs, and the plasma concentration−time profiles were followed for 12 h postadministration by HPLC. The absorption of uncomplexed drug from the capsules was rapid with a short biological half-life while sustained-release patterns were evident for absorption from the peracylated β-CD complexes. Interestingly, only the perbutanoyl derivative increased the area under the plasma concentration curve and produced elevated plasma levels for a sustained period (Figure 9). The mean residence times for the perbutanoyl and perhexanoyl derivatives were about twice those of the uncomplexed drug and the peracetyl complex, also suggesting a sustained-release action in this model.

While the differences in the *in vivo* behavior of the perbutanoyl and perhexanoyl derivatives were not addressed, the authors suggest that the sustaining effect exhibited with the perbutanoyl derivative is a result of both increased hydrophobicity and mucoadhesive properties. Nevertheless, these results suggest utility of such technology in sustained-release applications. Similar effects were observed with ethylated derivatives of β-CD for the oral administration in rats of diltiazem hydrochloride.[164] In these studies, DE-β-CD was shown to decrease the peak plasma concentration of diltiazem while sustaining its plasma concentration over a 36 h period.

With regard to delayed-release technologies with an immediate release (cyclodextrin driven) and a delayed-release component, Wang et al.[165] studied the *in vivo* behavior in dogs of modified-release oral dosage forms of nifedipine using an HP-β-CD complex as the immediate release portion of the tablet and (hydroxypropyl)cellulose (HPC) in the slow release portion of the tablet. On the basis of *in vitro* data, a double-layer tablet with 1 part by weight HP-β-CD and 3 parts by weight HPC was selected as an appropriate dosage form to compare to commercially available slow-release nifedipine preparations (Adalat). In limited studies these HP-β-CD containing tablets exhibited plasma level−time profiles comparable to those of the commercial tablets. The authors suggest that this technology may offer a more balanced oral nifedipine bioavailability with prolonged therapeutic effects.

In studies aimed at alleviating unwanted effects resulting from the low pH of the stomach, *O*-carboxymethyl-*O*-ethyl-β-CD (CME-β-CD) derivatives which exhibit pH dependent solubility behavior have been investigated. Recent reports on this technology have included *in vitro* studies and *in vivo* evaluation of diltiazem[166] and molsidomine[167] release in gastric acidity controlled dogs. The basis of the acid protection of a drug involves the limited solubility of the CME-β-CD derivative and its inclusion complex at pH values below the pK_a of CME-β-CD (estimated to be 3.7 based on pH−solubility data) such as those of the stomach. As the dosage form passes into an environment with higher pH (the upper small intestine), the CME-β-CD solubility increases as a result of ionization of the carboxylate moieties, thereby increasing drug dissolution, solubility, and release.

In the studies concerning diltiazem release, Uekama et al.[166] controlled the gastric pH of fasting beagle dogs with tetragastrin to obtain a high acidity level (less than pH 2) or omeprazole to obtain a low acidity level (higher than pH 6). The absorption of diltiazem following oral administration was retarded under high gastric acidity conditions ($t_{max} = 4.0 \pm 0.0$ h) while fast absorption was observed under low gastric acidity conditions ($t_{max} = 2.3 \pm 0.5$ h). The C_{max} and AUC values were similar for the two conditions. The authors also generated the deconvoluted plasma level−time curves (Figure 10) to accentuate the retarded diltiazem absorption observed in the high gastric acidity dogs. These *in vivo* results were in good agreement with *in vitro* release data measured using a pH changeable dissolution apparatus. Similarly, Horikawa et al.[167] studied the *in vitro* and *in vivo* release of molsidomine using the CME-β-CD technology and a fasted/nonfasted dog

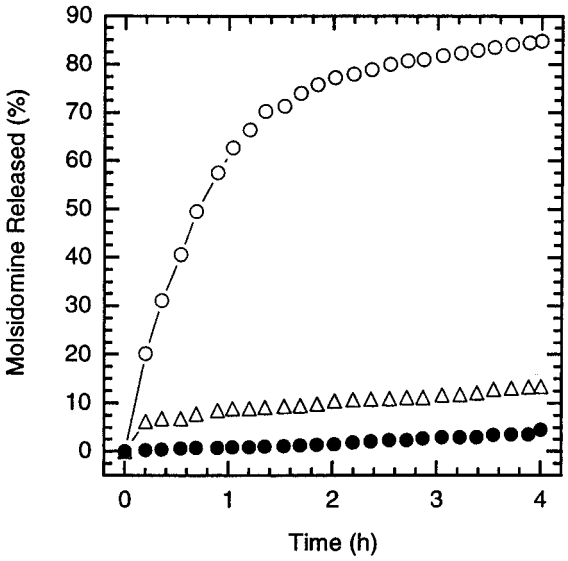


Figure 11—Release profiles of molsidomine from tablets containing its CME-β-CD complex (equivalent to 10 mg of molsidomine) in dissolution media of different pH values at 37 °C. Key: (●) JP XII first fluid (pH 1.2), (△) sodium acetate buffer (pH 4.0), (○) JP XII second fluid (pH 6.8). Reprinted with permission from ref 167. Copyright 1995 Royal Pharmaceutical Society of Great Britain.

model. As can be seen in Figure 11, the *in vitro* release profiles of molsidomine from tablets containing CME-β-CD exhibit the expected solution pH dependence. In the *in vivo* studies, the absorption of molsidomine was significantly retarded under high gastric acidity conditions under both fasted and nonfasted conditions (Figure 12). The delayed-release pattern, however, was more pronounced in the fasted dogs, probably as a result of lower dosage form motility and stomach emptying rates in the fasted dogs. As in the diltiazem studies, the AUC values were similar in the high and low gastric acidity dogs under the fasted and nonfasted conditions.

Decrease of Local Tissue Irritation or Masking of Objectionable Taste—Cyclodextrins and their derivatives may decrease local tissue irritation or mask objectionable taste through a decreased percentage of free drug resulting from the inclusion equilibrium. The work of Puglisi et al.[148] concerning the decreased incidence of gastric lesions resulting from oral administration in rats of 4-biphenylacetic acid as the β-CD or HP-β-CD complex has been previously reviewed. In earlier studies,[168] Publisi et al. made similar preliminary observations in the rat with this drug and β-CD.

Several studies concerning the reduction in gastric injury of piroxicam dosage forms containing β-CD versus the free drug have been reported.[169−171] Santucci et al.[169] investigated the acute gastroduodenal mucosa injury in healthy human volunteers following 14 day administration of 20 mg·day^{-1} of piroxicam as a β-CD complex or as free drug and 100 mg·day^{-1} of indomethacin. The authors also measured gastric potential differences (GPD) following a single oral dose of these formulations. Endoscopic examinations performed after the 14 day treatment demonstrated that the β-CD formulation was less gastrolesive than either piroxicam or indomethacin. The drop in GPD was statistically greater for piroxicam and indomethacin than for the β-CD complex, which had a GPD value similar to that of the placebo. Similar results were obtained by Nervetti et al.,[170] who investigated the gastric mucosal damage of piroxicam and its β-CD complex in patients with rheumatological disorders and a control group of healthy volunteers. In these studies, gastric mucosal damage was determined by comparing scintigraphy and endoscopy with scintigraphy and biopsy. The results show a significantly

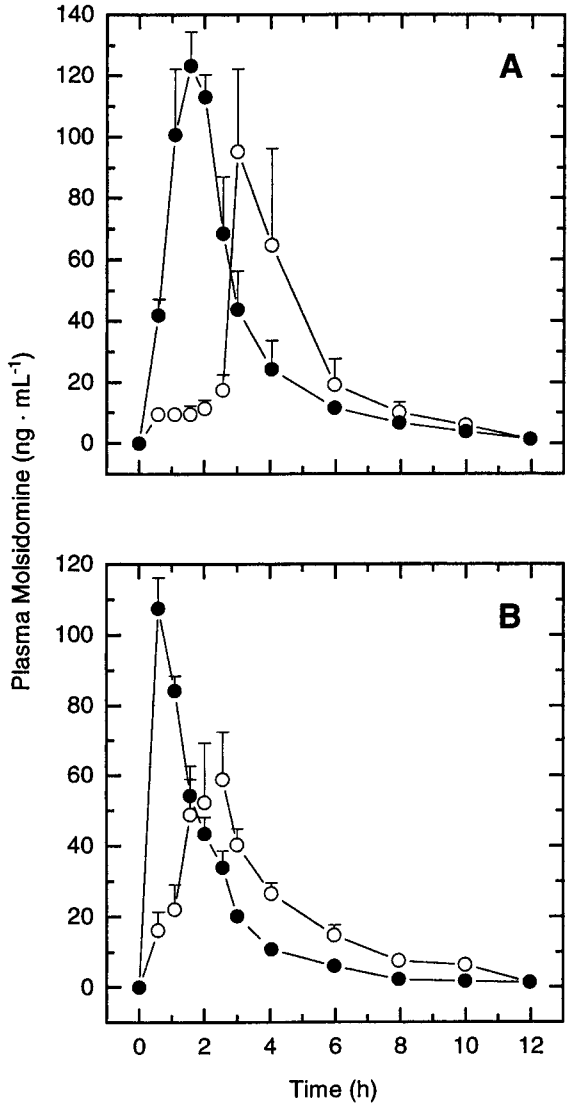


Figure 12—Plasma levels of molsidomine after oral administration of tablets containing its CME-β-CD complex (equivalent to 10 mg of molsidomine) in gastric acidity controlled dogs under fasted (A) and nonfasted (B) conditions. Key: (●) low gastric acidity dogs, (○) high gastric acidity dogs. Reprinted with permission from ref 167. Copyright 1995 Royal Pharmaceutical Society of Great Britain.

lower incidence of gastric lesions in patients who received piroxicam in the β-CD complex. Similar findings were reported by Patoia et al.[171] in healthy human volunteers. Collectively, these results indicate that β-CD complexes of piroxicam induce less gastric injury than the noncomplexed dosage forms.

In studies investigating the reduction in ulcerogenicity of naproxen by complexation with β-CD, Otero Espinar et al.[172] looked at the macroscopic damage to gastric mucosa of rats following oral administration of the complex or free drug. Damage was evaluated by five independent people from photographs of the animal stomachs following a 5 day dosing regimen. Points were given on a moving scale for reddened spots or petechiae, desquamations, and ulcers. A statistically significant decrease in ulceration was seen with the β-CD complex of naproxen versus the free drug. There was no difference between the animals treated with the complex and the control group. These results are in agreement with the work of Lin et al.[173] involving the gastrointestinal tolerance of (hydroxyethyl)cellulose hydrogels containing indomethacin as the free acid or the β-CD or HP-β-CD complex. Following daily oral administration of these hydrogels to rats for 3 days,

the animals were sacrificed and the visual examination of the number of lesions was conducted. The total number of lesions for 12 animals receiving the indomethacin hydrogel was 28 while the total lesions for each group of 12 animals receiving the β-CD or HP-β-CD were one and four, respectively.

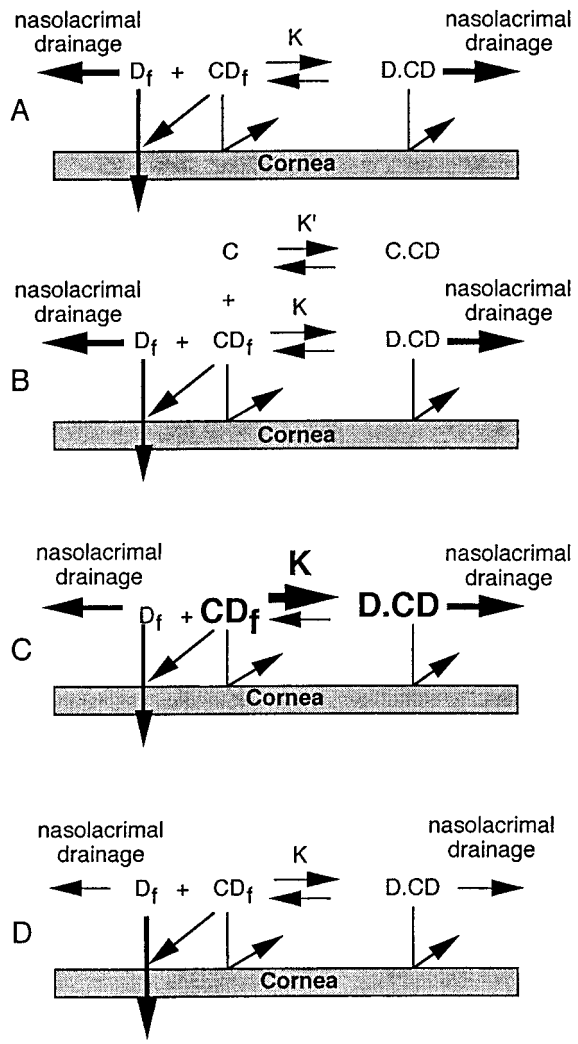
The bitterness of drug formulations may also be decreased by inclusion complexation. Miyaji et al.[174] reported on the increase in oral bioavailability in rabbits of fenbufen by complexation with α- or γ-CD. The cyclodextrin formulations increased the bioavailability of fenbufen by 2–3 times. As a side study, the authors subjected these formulations to a sensory panel test. Ten subjects qualitatively evaluated the bitterness of these formulations and classified them as either slightly, moderately, or markedly bitter. While both the free drug and γ-CD complex were ranked as markedly bitter, the α-CD complex was classified as being only slightly bitter.

In summary, the use of cyclodextrins for oral applications is appropriate in cases of dissolution rate limited drug absorption. The use of cyclodextrins in such applications, however, is limited to low-dose drugs with large stability constants due to mass limitations for oral dosage units. A more complex regulatory problem is the clinical use of cyclodextrins to increase bioavailability through cyclodextrin-associated increases in membrane permeability. Such applications will be closely scrutinized in the approval process, and their future clinical usefulness remains unknown.

Ophthalmic Applications of Cyclodextrins

Summary of Reviews to Date—van Doorne[11] has recently reviewed the interactions between cyclodextrins and ophthalmic drugs. As pointed out by van Doorne,[11] the use of cyclodextrins in ophthalmic preparations has received little attention. Recently, however, both the strengths and weaknesses of the use of cyclodextrins in this application have been recognized. An obvious concern with the use of cyclodextrins is whether they have any effect on the integrity of the cornea. A key paper on this issue by Jansen et al.[175] demonstrated through the use of slit lamp biomicroscopy (SLB) and scanning electron microscopy (SEM) that dimethyl-β-cyclodextrin (DM-β-CD) after both single and multiple applications was not a suitable vehicle for ophthalmic formulations. DM-β-CD at both 5% and 12.5% caused conjuctival hyperemia and desquamation of corneal epithelial cells with the effects becoming severe at the high dose. These observations are consistent with the ability of DM-β-CD to effectively extract cholesterol, phospholipids, and proteins from biological membranes and cause lysis of red blood cells. In the same study, HP-β-CD at 12.5% did not cause any alterations in the corneal tissues.

It is generally assumed that only free drug (D_f), not the cyclodextrin complex of a drug, penetrates across biological membranes. This is illustrated in Scheme 3A. The formulator needs to understand the factors which affect corneal absorption/transport when considering how cyclodextrins might be useful in ophthalmic drug delivery. There is only about 7–12 μL of precorneal tear fluid, and most traditional droppers place 25–40 μL of fluid in the eye. Minimal dissociation of drug from the complex should occur through simple dilution, and rapid nasolacrimal drainage should quickly sweep excess fluids from the precorneal area. Of course, as suggested above, the CD could influence corneal permeability either by a small reversible temporal change to the membrane or through actual corneal tissue damage (unacceptable). The precorneal fluid is a complex milieu with components that can compete with the drug for cyclodextrin binding (Scheme 3B). By interacting with free cyclodextrin, competing agents (C) help cause dissociation of the



Scheme 3—Scheme illustrating various pathways for transcorneal drug absorp ion and loss in the presence of cyclodextrins. A: Equilibrium and kinetic steps assumed to be critical for the transport and delivery of ophthalmic drugs in the presence of cyclodextrins. B: Competition for drug–cyclodextrin complex dissociation can come from precorneal components such as C. C: Drug transport can be inhibited by the presence of excess cyclodextrin which produces a higher nasolacrimal excretion of the complex. D: Nasolacrimal drainage may be slowed in he presence of viscosity-inducing agents allowing for greater residence time in the precorneal area.

cyclodextrin complex of the drug (D.CD). Also, if the cornea is very permeable to the drug, the cornea acts as a sink, with drug absorption resulting in further dissociation of the complex.

The importance of this dynamic process will become obvious as we proceed. If large excesses of cyclodextrin are present, they can lower the free fraction of drug and increase the fraction of drug in the complex form, thereby lowering the effectiveness of the agent (Scheme 3C) by pushing the equilibrium to the right, thus increasing the probability of greater nasolacrimal excretion of the complex. The authors are familiar with an unpublished report where a cyclodextrin, present in excess, was used to stabilize an ophthalmic drug. At the concentrations needed to effect stabilization, the cyclodextrin formulation of the drug was completely ineffective.

Cyclodextrins and Corneal Permeability—Cyclodextrins may increase the ophthalmic delivery of drugs through multiple routes. One route would be to increase the solubility of poorly water soluble drugs. A second route would be to alter corneal permeability as illustrated in Scheme 3A. If

Table 3—Effects of SBE4-β-CD, HP-β-CD, DM-β-CD, and BAC on Pilocarpine (0.516 mM) Permeability through an Isolated Bovine Cornea *in Vitro* (Mean ± SE)[176]

Treatment	Permeability Coefficient ($\times 10^{-6}$) (cm·s^{-1})	*n*
Pretreatment[a]		
None	4.05 ± 0.27	10
Buffer	4.11 ± 0.28	6
SBE4-β-CD (58 mM, 10.0%)	3.67 ± 0.32	5
HP-β-CD (58 mM, 7.8%)	4.26 ± 0.22	3
DM-β-CD (58 mM, 7.7%)	6.72 ± 0.50[b]	4
BAC (0.12%)	15.60 ± 0.61[b]	3
Cyclodextrin in donor side[c]		
SBE4-β-CD (1.86 mM, 0.32%)	3.92 ± 0.45	4
HP-β-CD (1.86 mM, 0.25%)	3.55 ± 0.18	3

[a] Pretreated with solutions (5 mL, 37 °C, 30 min). [b] Significantly different from the value for "none" at 95% confidence (ANOVA, Fisher's PLSD test). [c] Cyclodextrin was added to the donor side with pilocarpine.

corneal permeability is increased, the change may be transitory or it may result from permanent corneal tissue damage. Järvinen et al.[176] recently reported on the permeability of pilocarpine, a relatively polar drug, through an isolated bovine cornea *in vitro*. They argued that an increase in corneal permeability would suggest the possibility of corneal damage while no increase would suggest little or no corneal damage. In their study, freshly harvested corneas were pretreated for 30 min at 37 °C with GBR buffer or GBR buffer containing either 58 mM SBE4-β-CD (10%), HP-β-CD (7.8%, Encapsin), DM-β-CD (7.7%), or 0.12% benzalkonium chloride (BAC, positive control). Table 3 is a summary of their findings. Both DM-β-CD and the BAC significantly increased the permeability of pilocarpine through the bovine cornea, consistent with the known damaging potential of BAC at this dose level and with the microscopic observations of Jansen et al.[175] of the corneal damage caused by 5 and 12.5% DM-β-CD. Pretreatment with SBE4-β-CD and HP-β-CD had no effect on pilocarpine permeability.

Järvinen et al.[176] data, therefore, brought into question whether a nondamaging cyclodextrin should alter the penetration of a polar drug like pilocarpine across the cornea. Freedman et al.[177] had reported increased miotic response to 50 μg of pilocarpine in 5% HP-β-CD as compared to pilocarpine alone. Maximal effect of the cyclodextrin was observed when the pilocarpine to HP-β-CD ratio was 1:1. They concluded that the increased response may have been due to the shifting of the equilibrium between the ionized and un-ionized states of pilocarpine because, like Jansen et al.,[175] they did not observe microscopic changes in the cornea treated with HP-β-CD. Additionally, no changes were observed in transcorneal resistance and potential or short-circuit current using electrophysiological measurements in isolated corneas. Järvinen et al.[176] repeated some of the studies of Freedman et al. using a more precise method for determining changes in pupillary diameter after the administration of pilocarpine with and without SBE4-β-CD and HP-β-CD and showed that neither cyclodextrin significantly altered the miotic response to pilocarpine. Therefore, the observations of Freedman et al. remain unexplained.

Järvinen et al.[176] also studied the miotic response to pilocarpine, a drug which only weakly associates with cyclodextrins ($K < 200$ M^{-1}), to see if excessive cyclodextrin reduces the miotic response to pilocarpine. Figure 13 shows plots of the reduction of pupillary diameter AUC (%·h) versus SBE4-β-CD concentration for 0.5% pilocarpine (24 mM) at pH 4.5 and 7.0. At pH 4.5, a reduction in AUC values with increasing concentration of the cyclodextrin was more pronounced than that seen at pH 7.0. While the cyclodextrin did not enhance

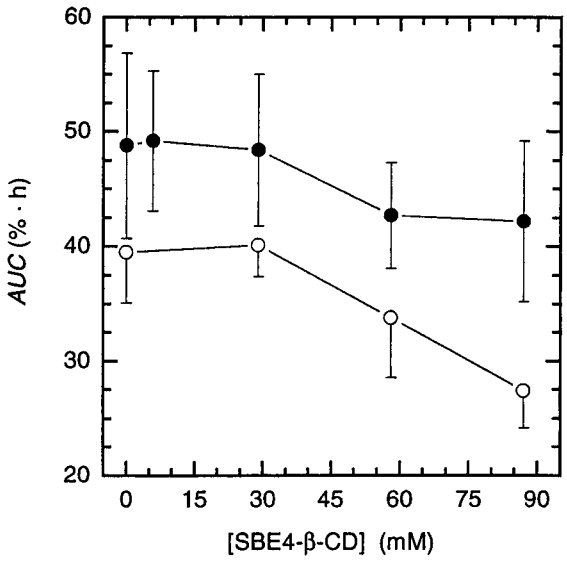


Figure 13—Reduction in pupillary diameter AUC values after ocular, unilateral administration of pilocarpine solution (0.5%, 24 mM, 25 μL) at pH 4.5 (○) and 7.0 (●) in the presence of increasing SBE4-β-CD concentrations.[176]

the activity of pilocarpine, it only reduced activity when the ratio of cyclodextrin to pilocarpine was greater than 1:1.

Reer et al.[178] recently reported on the *in vitro* corneal permeability of diclofenac (0.1%, a weak acid) as well as the hemolysis of red blood cells in isotonic phosphate buffer (PBS) of pH 7.4, with and without added HP-β-CD. Corneal permeability of diclofenac, 0.1% at pH 7.4, was highest from diclofenac in BRS-HEPES buffer and decreased, as expected, in the presence of various cyclodextrins (7 mM) including HP-β-CD. Diclofenac in a 0.1% commercial product, Voltaren ophtha, exhibited the poorest permeability presumably, due to the presence of a surfactant, polyoxyethylene-35-castor oil. The permeability with HP-β-CD was enhanced when the pH of the solution was lowered, presumably because a greater fraction of the diclofenac was present in its un-ionized state.

Diclofenac in PBS caused 50% hemolysis of red blood cells at 10.8 mM (±0.06) and 40.7 mM in the absence and presence of HP-β-CD, respectively. HP-β-CD caused 50% hemolysis at 61.9 mM (±1.3). At 0.1% (3.14 mM), diclofenac in pH 7.4 PBS with and without HP-β-CD showed no hemolysis, whereas the Volatren formula caused 22.7% (±2.2%) hemolysis presumably due the presence of the surfactant.[178]

Ophthalmic Delivery of Insoluble Drugs—An area where cyclodextrins may have a significant therapeutic benefit is the solubilization of drugs intended for ophthalmic use. Poorly water soluble drugs are ophthalmically administered as suspensions or in ointment dosage forms. Both delivery systems are less than ideal, the former leading to irritation if the particles are too large and the latter leading to blurred vision. Both delivery systems can also lead to erratic availability of the drug.

Cyclosporin A (CsA) is a very poorly water soluble immunosuppresive drug that has been shown to be useful in organ transplantation, including corneal allografts,[179] and is being considered for the treatment of dry eye. Because of its insolubility, CsA has been administered in a variety of vehicles including ointments and emulsions and as oily solutions or suspensions.[180] α-CD has been used to solubilize CsA, and solutions as high as 0.075% are achievable with 80 mg·mL^{-1} α-CD.[181] However, α-CD, with and without CsA at 80 mg·mL^{-1}, did cause observable changes to corneal tissues including changes in the microvilli of the epithelial cells. A concentration of 40 mg·mL^{-1} CsA caused some mild changes while at less than 40 mg·mL^{-1} the corneas appeared to be

Table 4—Relative Bioavailability of Dexamethasone and Dexamethasone Acetate from 0.1% Suspensions[a] and 0.1% Solutions with HP-β-CD[b] [183]

Tissue	Rel Bioavailability		
	DX-HP-β-CD/DX	DXA-HP-β-CD/DXA	DXA-HP-β-CD/ DX-HP-β-CD
Conjunctiva	2.1	1.7	2.3
Cornea	1.9	1.8	2.3
Iris	1.5	1.8	2.6
Aqueous humor	1.4	2.0	2.6

[a] DX and DXA, respectively. [b] DX-HP-β-CD and DXA-HP-β-CD, respectively.

unaffected. In both *in vitro* diffusion experiments using isolated corneas and a number of *in vivo* experiments, the activity of CsA formulated with α-CD appeared to be superior to that from other dosage forms. It remains to be seen whether α-CD proves useful as a vehicle for CsA delivery.

Although prostaglandins have proven useful for the treatment of ocular hypertension, most prostaglandins are sparingly water soluble and have been found to be irritating to the cornea. Wheeler[182] has claimed that various cyclodextrins, especially HP-β-CD, can be used both to increase the effectiveness of the prostaglandins and to decrease the incidence and severity of ocular surface hyperemia thus facilitating the clinical use of the prostaglandins.

Perhaps the best examples of the use of cyclodextrins with poorly water soluble drugs have been the work with steroids, especially dexamethasone and its acetate ester, dexamethasone acetate. Usayapant et al.[183] studied the solubility, chemical stability, and ophthalmic delivery of dexamethasone and dexamethasone acetate in the presence and absence of HP-β-CD. Usayapant et al.[183] claim a solubility of 63 $\mu g \cdot mL^{-1}$ (0.16 mM) for dexamethasone (DX) and 10 $\mu g \cdot mL^{-1}$ for dexamethasone acetate (DXA) in the absence of HP-β-CD and solubilities as high as approximately 8 $mg \cdot mL^{-1}$ and 3 $mg \cdot mL^{-1}$ for dexamethasone and dexamethasone acetate in the presence of 0.1 M HP-β-CD. HP-β-CD was also shown to increase the chemical stability of both dexamethasone and its acetate ester.

To assess ophthalmic availability of dexamethasone and dexamethasone acetate, Usayapant et al.[183] prepared 0.1% suspensions of each drug and 0.1% solutions with 5% HP-β-CD for dexamethasone and 8% for the acetate ester. Aqueous humor, cornea, iris, and conjunctiva concentrations were determined postinstillation of 25 μL of the suspensions or the solutions. AUCs for the different tissues and from the different formulations were used to assess relative bioavailabilities. The results are presented in Table 4. The bioavailabilities of dexamethasone from dexamethasone and from its acetate ester are approximately double from the HP-β-CD solutions as compared to the suspensions. Shorter t_{max} values were also obtained from the solutions as compared to the suspensions. The authors concluded that simple dilution resulting in dissociation of the complexes could not account for the increased bioavailability from the solution but that lipids and other components in the precorneal fluids might facilitate dissociation and release. The higher cornea permeability and rapid conversion of the acetate ester to dexamethasone probably accounts for the better performance of the acetate ester.

Loftsson et al.[184] in studying the effect of (hydroxypropylmethyl)cellulose (HPMC) on the complexation of dexamethasone with HP-β-CD also evaluated the relationship between HP-β-CD concentration and dexamethasone flux through a semipermeable cellophane membrane (with and without added HPMC). Sink conditions were maintained on the receptor side by the use of 5% HP-β-CD. A plot of flux versus

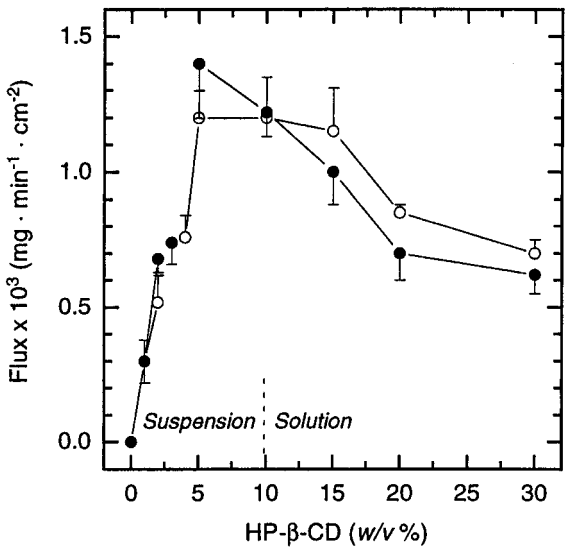


Figure 14—Flux of dexamethasone across a semipermeable cellophane membrane from a 0.5% dexame hasone suspension or solution as a function of HP-β-CD concentration both in the absence (○) and in the presence (●) of 0.1% (hydroxypropylmethyl)cellulose. Reprinted with permission from ref 184. Copyright 1994 Elsevier.

HP-β-CD concentration is shown in Figure 14. These observations offer a reasonable explanation for the increased delivery of dexamethasone from a HP-β-CD solution of dexamethasone versus a dexamethasone suspension. Referring back to Scheme 3C, the solubility of total dexamethasone can be increased by increasing concentration of HP-β-CD. However, if the dexamethasone is added to the donor side of a semipermeable membrane and the receptor phase is operating under sink conditions, the dexamethasone will readily cross the membrane and be replaced through dissociation of the complex. Thus, while the free fraction of drug from a saturated suspension of drug would be the same as that from a saturated suspension of the drug in the presence of HP-β-CD, the excess complexed drug acts as a source of free drug through the dissociation process. *In vivo*, not only might the cornea and other tissues act as a sink, but as discussed with Scheme 3B, other components from the precorneal area can help cause dissociation of the complex through a competitive process. Figure 15 is a plot of dexamethasone aqueous humor concentration versus time after administration of a 1.3% dexamethasone solution with 20% HP-β-CD and 0.1% HPMC (also containing 0.02% BAC and 0.1% EDTA). Data from a 0.1% dexamethasone formulation available as a commercial alcoholic suspension (Maxidex) are also included. Clearly the HP-β-CD solution outperformed the suspension although the dose difference probably also contributed to the higher aqueous humor levels.

Loftsson et al.[185,186] performed similar studies in an attempt to increase the topical effectiveness of two carbonic anhydrase inhibitors, acetazolamide and ethoxyzolamide, using HP-β-CD. The authors concluded that HP-β-CD increased the ocular bioavailability of acetazolamide by solubilizing the acetazolamide and keeping it at the corneal barrier. The results confirmed that HP-β-CD itself does not cross the corneal membrane.

Pate et al.[187] were able to study the effect of arachidonylethanolamide, a sparingly water soluble drug, on the decrease of intraocular pressure in normotensive rabbits. Although the study was designed to test the activity of arachidonylethanolamide, the effect of the drug would have been difficult to determine because of its insolubility. HP-β-CD was able to provide a solution form of the drug.

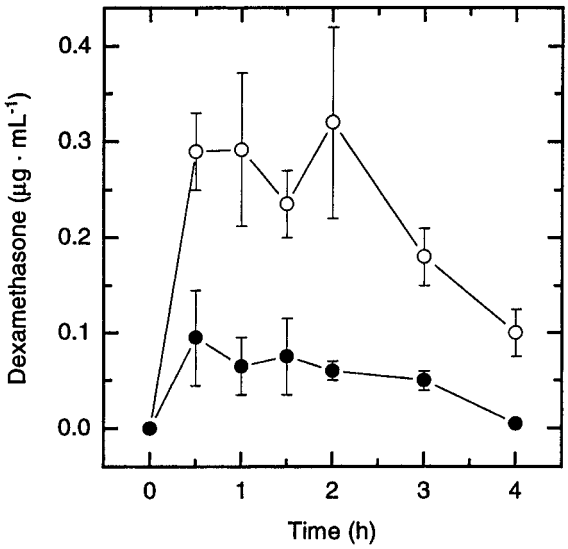


Figure 15—Aqueous humor dexamethasone concentration versus time after administration of 1.3% dexamethasone in an HP-β-CD solution (○) or a 0.1% dexamethasone alcoholic suspension (●). Reprinted with permission from ref 184. Copyright 1994 Elsevier.

Decreased Irritation of Ophthalmic Drugs—Many drugs are clearly irritating when applied to the precorneal area. Depending on severity, irritation can doom the development of a potentially useful ophthalmic drug. In other cases, mild irritation might decrease patient compliance with a product. For example, the anti-inflammatory agent ketorolac, a drug useful for the treatment of allergic conjunctivitis, causes stinging and burning in up to 40% of users. Unfortunately, this effect was not noted in the New Zealand white rabbit during animal testing. In some unpublished work, the authors measured the eye closure and half-closure time as well as the blinking rate in rabbits after administration of a 0.5% (13.3 mM) pH 7.4 ketorolac tromethamine salt, with and without added SBE4-β-CD (13.3 mM) in a masked study. The results are displayed in Table 5. Although the effects were small, there did appear to be an effect from the cyclodextrin on both the blinking rate and the eye half-closure times. These results are consistent with the work of Reer et al.,[178] who also suggested on the basis of *in vitro* experiments that HP-β-CD might decrease the irritation of diclofenac, another nonsteroidal anti-inflammatory drug. Masuda et al.[111] in a comprehensive study of the protective effects of cyclodextrins showed that β-CD was able to offer some protection to the irritancy of flurbiprofen after ophthalmic administration to both rabbits and humans.

A more dramatic effect of cyclodextrins in decreasing ophthalmic irritation has been shown in a recent series of papers. Järvinen et al.[188] studied the effect of SBE4-β-CD on eye irritation and miotic response in albino rabbits of an ophthalmically applied lipophilic pilocarpine prodrug, *O,O′*-dipropionyl (1,4-xylylene)bis(pilocarpate). They compared the miotic response of 12−24 mM prodrug solutions to 163 mM pilocarpine (3.4%) and showed that the prodrug had a decreased peak miotic intensity but an increased time to reach peak response. The 24 mM prodrug solution (equivalent to 1% pilocarpine) gave the same 0−6 h AUC for the change in pupillary diameter as the 163 mM pilocarpine. Unfortunately, this promising prodrug caused extreme irritation.

Irritation intensity and duration of action of the prodrug and the pilocarpine solution were estimated in this masked study by determining eye closure and half-closure times as well as mucoid discharge scored on a scale of 0−2 with 0 being normal (no lacrimation), 1 being slight discharge (any amount different from normal, clear discharge), and 2 being strong discharge (intense lacrimation and milky discharge). Figure 16 is a plot showing the effect of pH and the presence and absence of SBE4-β-CD (as well as various controls) on eye closure and half-closure time of the prodrug. At SBE4-β-CD to prodrug ratios of as low as 1:1, SBE4-β-CD clearly decreased the ophthalmic irritation to the prodrug. At 0.25 h after administration of 12 and 24 mM prodrug, the mucoid discharge score was 0.2 ± 0.2 and 0.8 ± 0.2, respectively, while 1 h after administration, the scores were 0.7 ± 0.3 and 1.8 ± 0.2, respectively. For 163 mM pilocarpine and the prodrugs in the presence of SBE4-β-CD, the scores were 0.0 ± 0.0 at both time points, i.e., SBE4-β-CD completely abolished the mucoid discharge response to the prodrug. At higher SBE4-β-CD to prodrug ratios (>1:1), the miotic response (as measured by AUC and peak response values) did decrease, suggesting that while the cyclodextrin decreased the irritation due the prodrug, it also decreased probable postcorneal delivery of pilocarpine.

An interesting question is whether increasing the residence time of the drug and complex in the precorneal area would result in an improved response selectively from the irritation. Suhonen et al.[189] studied the same pilocarpine diester prodrug (equivalent to 0.5%) with and without HP-β-CD and with and without the viscosity-inducing agent HPMC at a formulation pH of 5.0. They also studied other variables including buffer strength and concluded that in the absence of HP-β-CD increasing buffer strength led to a decrease in miotic response while an increase in viscosity resulted in an increase in the intensity of the miotic response as well as the degree of irritation. HP-β-CD alone decreased both the ocular delivery of pilocarpine from the prodrug and the irritation. The combination of HP-β-CD and HPMC was able to return the miotic response while maintaining the irritation at an intensity level comparable to that seen with a commercial pilocarpine solution.

In a paper published recently by Jarho et al.,[190] a similar study compared the effect of SBE7-β-CD and HP-β-CD on the intensity and duration of action, as well as irritation, of bispilocarpine prodrugs. They also looked at increasing the viscosity of the solutions with poly(vinyl alcohol) (PVA) to compensate for the decreased activity seen at higher cyclodextrin concentrations. The miotic AUC values to 6 h from 12 mM prodrug, without and with 24 or 36 mM SBE7-β-CD, were 85.5 ± 14.5%·h, 64.1 ± 13.4%·h, and 40.7 ± 13.4%·h, respectively. These results demonstrate again that higher precorneal concentrations of cyclodextrins can decrease the response to precorneally administered drugs. Ocular delivery of the prodrug was improved as indicated by an AUC value of 97.5 ± 15.5%·h when the viscosity of the SBE7-β-CD prodrug solution was raised with PVA (20−30 mg·mL^{-1}) without inducing irritation. The eye irritation due to the SBE7-β-CD prodrug solution with PVA was comparable to that of isotonic saline solution. As illustrated in Scheme 3D, the PVA probably slows drainage (as indicated by the size of the arrows), thus increasing the contact time of both the free drug and the complex with the corneal tissue.

Limitations on the Use of Cyclodextrins for Ophthalmic Drugs—Obviously cyclodextrins have no place in ophthalmology if they cause or contribute to tissue damage. To be considered by regulatory agencies, the safety of the cyclodextrins must be clearly established. This is not only with respect to corneal and other ophthalmic tissues, but because a significant portion of an ophthalmically applied fluid drains via the tear duct into the nasopharangyl cavity and on into the GI tract, the oral safety of the cyclodextrin must be verified.

Cyclodextrins are also capable of strong interactions with various preservatives such as benzalkonium chloride (BAC) and the parabens.[191−193] To be used in multidose preserved

Table 5—Blinking Rate and Eyelid Closure Times after Topical Administration of 0.5% Ketorolac Solutions (Made Isotonic with NaCl, Mean ± SE; $n = 6$)

Solution	Blinking Rate[a] (min^{-1})	Eyelid Closure (s)[b]	
		Closed	Half-Open
13.3 mM (2.3%), SBE4, pH 7.4	0.2 ± 0.2 (1)	0 ± 0 (0)	0 ± 0 (0)
13.3 mM (0.5%), ketorolac as tromethamine salt, pH 7.4	1.7 ± 0.5 (10)	0 ± 0 (0)	38 ± 18 (225)
13.3 mM (0.5%), ketorolac as tromethamine salt, + 13.3 mM SBE4, pH 7.4	1.2 ± 0.5 (7)	0 ± 0 (0)	10 ± 5 (60)

[a] The figure in parentheses represents the sum of blinks measured over the first minute. [b] The figure in parentheses represents the sum of duration of eye closure.

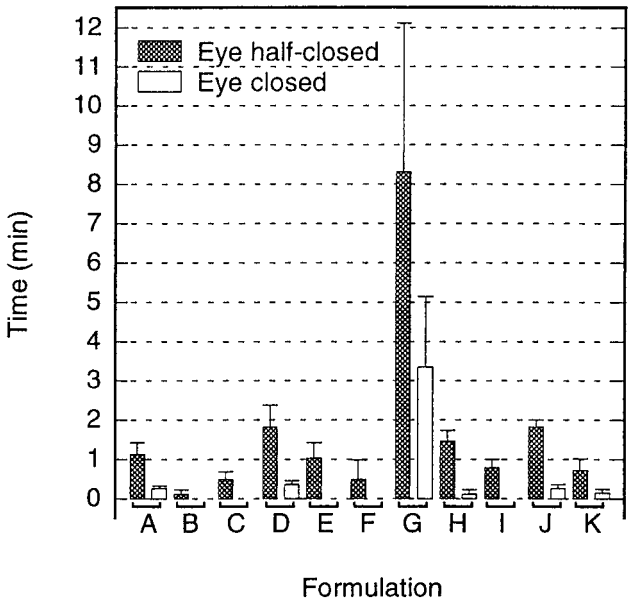


Figure 16—Duration of eyelid closure after ocular administration of 25 μL of pilocarpine (Oftan Pilocarpin) or pilocarpine prodrug (*O,O′*-dipropionyl (1,4-xylylene)-bis(pilocarpate)) (PRO) in the presence or absence of SBE4-β-CD (SBE) in albino rabbits (mean ± SE, $n = 5–6$). Key: (A) Oftan Pilocarpin (3.4%), (B) 58 mM SBE (pH 5.0), (C) 0.9% NaCl (pH 5.0), (D) 12 mM PRO (equivalent to 0.05% pilocarpine, pH 5.0), (E) 12 mM PRO and 14.5 mM SBE (pH 5.0), (F) 12 mM PRO and 29 mM SBE (pH 5.0), (G) 24 mM PRO (equivalent to 1.0% pilocarpine, pH 5.0), (H) 24 mM PRO and 29 mM SBE (pH 5.0), (I) 24 mM PRO and 58 mM SBE (pH 5.0), (J) 24 mM PRO and 29 mM SBE (pH 6.0), (K) 24 mM PRO and 58 mM SBE (pH 6.0). Reprinted with permission from ref 188. Copyright 1995 Liebert.

preparations, new preservatives that do not interact with the cyclodextrins may have to be identified. Matsuda et al.[191] did note, however, that the longer chain parabens, propyl and butyl, were solubilized by HP-β-CD so that it my be possible to use a combination of HP-β-CD and hydrophobic longer chain alkyl parabens for the preservation of liquid formulations. This does not preclude the use of cyclodextrins in unit dose formulations or some of the newer technologies being considered for nonpreserved, multidose containers.

Nasal Applications of Cyclodextrins

Summary of Reviews to Date—The use of cyclodextrins to improve the delivery of drugs after nasal administration has been quite extensively reviewed by Uekama et al.[25] as part of a larger review. Many of the principles that were applied to ophthalmic drug delivery may also be applied to nasal delivery. Improved nasal delivery can be attributed to changes in nasal mucosa permeability (an enhancer effect by the cyclodextrin), alterations in drug solubility, and in the case of some prodrugs and peptides, a change in the metabolism rate of the drugs at the site of delivery. Unlike solution administration to the precorneal area, there is probably greater contact time between the drug or the cyclodextrin complex of the drug and the nasal mucosal surface to allow for greater dissociation.

Cyclodextrins and Nasal Mucosa Permeability—In some unpublished results, Benelli et al.[194] studied the permeability of fluorescein and FITC-dextrans through freshly excised bovine nasal tracheal epithelium. The authors also measured the potential difference and short-circuit current and utilized histological studies to determine changes in the bovine nasal tracheal epithelium to assess damage in the presence of various cyclodextrins. DM-β-CD dramatically enhanced the permeability of the larger molecular weight FITC-dextrans compared to that of fluorescein whereas HP-β-CD had no statistically significant effect on the permeability of any of the fluorophores. SBE4-β-CD did not affect fluorescein permeability but decreased the permeability of one of the higher molecular weight FITC-dextrans. Consistent with the promoting effect of DM-β-CD, 5% and lower levels of DM-β-CD caused an increase in the release of the intracellular marker lactate dehydrogenase (LDH) when tissues were incubated for 5 h with this cyclodextrin. The release of LDH corresponded to extensive cell damage and erosion of the epithelium as determined from light microscopy studies. Irreversible changes in the electrophysiology of the tissue was noted with DM-β-CD. None of the other cyclodextrins studied, HP-β-CD, SBE4-β-CD, and SBE7-β-CD, had any adjuvant effect on nasal tracheal epithelium permeability or demonstrated any measurable changes in the histology and electrophysiology of the tissue or resulted in any LDH release.

Cyclodextrins and Nasal Mucosa Damage—Others have reviewed the effect of cyclodextrins on nasal drug delivery and issues associated with their possible nasal toxicity. Krishnamoorthy et al.[195] reported on the mucotoxicity of several cyclodextrins using a number of markers including the release of total protein, total phosphorus, and the intracellular enzymes LDH and alkaline phosphatase after an *in situ* nasal perfusion study in rats. The rank order damage to the mucosa was found to be DM-β-CD > M-β-CD (monomethyl-β-CD) > HP-β-CD > HE-β-CD (hydroxyethyl-β-CD) ≥ SBE4-β-CD. They concluded that the rank order of toxicity was related to the hydrophobicity of the cyclodextrin and the ability of the cyclodextrins to leach cholesterol and phospholipids from membranes. The same group had also earlier evaluated various cyclodextrins with regard to their ability to promote the nasal delivery of the protein insulin.[196] The promoting order of the cyclodextrins was DM-β-CD > α-CD > β-CD > HP-β-CD ≥ γ-CD. The promoting order was similar to the order seen in damage potential.

Marttin et al.[197] used a novel approach to determine the effects of various cyclodextrins on their absorption promotion potential in rats. Apart from determining the membrane release of various markers from membranes during an *in situ* nasal perfusion study in rats, they also measured the release into the perfusate of an FITC-albumin marker that had been injected into the rats prior to the nasal perfusion experiment. All the cyclodextrins studied (DM-β-CD, 2% and 5%; RM-β-CD, randomly methylated methyl-β-CD, 2%; and HP-β-CD, 5%) resulted in an elevation of cholesterol and FITC-albumin release with the rank order being DM-β-CD (5%) ≫ RM-β-CD (5%) > DM-β-CD (2%) > HP-β-CD (5%). All the cyclodextrins were much less damaging than the surfactants

sodium glycocholate and laureth-9 and the phospholipid L-α-lysophosphatidylcholine.

The authors concluded that solutions of 2% or less of DM-β-CD were suitable for promoting the absorption of polar drugs while producing minimal damage. Therefore, this group as well as others have focused on the use of DM-β-CD for the nasal delivery of a number of agents even though all the results suggest that some changes in nasal membranes occur even at low level exposure to this cyclodextrin derivative. Matsubara et al.[198] claim in their study that DM-β-CD facilitated delivery of buserelin, a luteinizing hormone-releasing hormone (LHRH) agonist, and that the irritation score from even 80 mM was acceptable. They quote that the results of a 6 month study show that DM-β-CD in concentrations up to 6% was well tolerated.

Cyclodextrins as Nasal Absorption Promoters—The oral systemic delivery of proteins and peptides to the body presents a considerable challenge because of their poor permeability, rapid metabolism in the luminal contents of the GI tract, and further presystemic metabolism in the enterocytes and liver. Like other tissues the nasal mucosa is readily permeable to lipophilic drugs and poorly permeable to larger molecular weight polar substances like proteins and peptides. Absorption promoters must be employed to effect delivery of polar substances, and in the case of proteins and peptides, their rapid metabolism must also be inhibited.

With respect to inhibiting the metabolism of proteins and peptides, a number of studies have produced interesting results. Irwin et al.[199] studied the stability of leucine enkephalin (YGGFL) and one of its major metabolites destyrosine leucine enkephalin (GGFL) in nasal tissue homogenates and nasal wash fluid. Like others earlier they found that these peptides were rapidly inactivated by these tissues and fluids. β-CD (25-fold excess) inhibited the degradation of YGGFL by leucine aminopeptidase (LAP) and glycylglycylphenylalanine (GGF) in the presence of carboxypeptidase (CAP). Chun and Chein[200] studied the metabolic inactivation and the effects of various inhibitors of methionine enkephalin in various rabbit mucosal extracts including that of the nasal tissue. HP-β-CD at concentrations of 5, 10, and 20% was found to inhibit the metabolic inactivation of methionine enkephalin by a rabbit nasal mucosa extract by 1.8-, 2.8-, and 4.1-fold, respectively.

Recently, Matsubara et al.[198] studied the nasal delivery of an agonist of LHRH, buserelin acetate, in rats. As part of their study they evaluated the protection of buserelin proteolysis by nasal mucosal homogenates by various cyclodextrins (80 mM). The order of inhibition was G_2-β-CD (diglucosyl-β-CD) ≈ DM-β-CD > HP-β-CD > DM-α-CD > α-CD > HP-α-CD ≈ β-CD > CM-β-CD. If the data for G_2-β-CD was excluded from the analysis, there was a reasonable correlation between the binding constant of the cyclodextrins with buserelin via interaction with the hydrophobic amino acids (tryptophan and tyrosine) and the inhibition of metabolism. G_2-β-CD was more effective than expected on the basis of its interaction constant with buserelin. The same research group had shown earlier that cyclodextrins can reduce the activity of leucine aminopeptidase in rat nasal homogenates. In the study with buserelin, Matsubara et al.[198] concluded that the nasal absorption promoting ability of DM-β-CD correlated more with its ability to promote membrane transport than its ability to inhibit the inactivation of buserelin.

The ability to deliver insulin and other proteins or peptides via the nasal route is thought to be highly desirable. Therefore, most studies on the effect of cyclodextrins on nasal delivery have focused on their potential to promote the delivery of this important class of drugs. Nasal delivery places the drug into systemic circulation thus bypassing presystemic metabolism that is thought to be a limiting factor in the efficient oral delivery of the protein or peptide.

Table 6—Systemic Bioavailability (±SE) of Insulin, 10 U·kg^{-1}, in Rats after Nasal Administration with and without Cyclodextrins (8 μM·kg^{-1})[150]

Cyclodextrin	Rel Bioavailability (*F*%)[a]
No cyclodextrin	0.2 ± 0.7
α-CD	12.3 ± 2.2
DM-α-CD	15.3 ± 2.7
HP-α-CD	5.1 ± 2.7
β-CD	20.9 ± 3.9
DM-β-CD	23.7 ± 3.9
HP-α-CD	3.9 ± 3.4

[a] Bioavailability compared using AUC values to insulin administered iv (5 U·kg^{-1}).

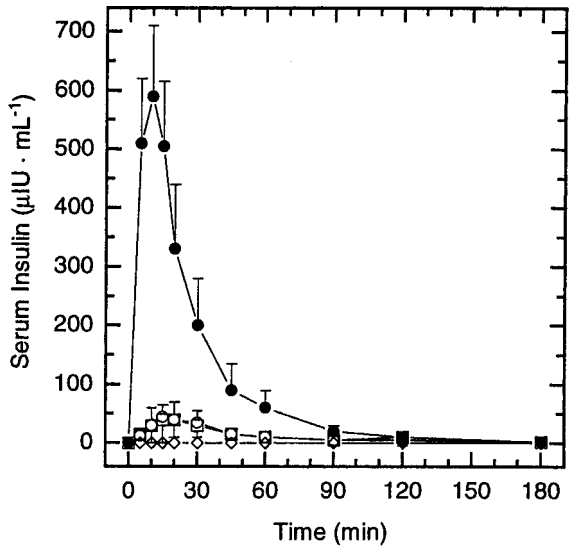


Figure 17—Serum insulin (μU·mL^{-1}, ±SD, $n = 6$) concentrations versus ime following intranasal administration of placebo (◇), an insulin and DM-β-CD solution (□), an insulin and lactose powder (○), and an insulin and DM-β-CD powder (●). Reprinted with permission from ref 205. Copyright 1993 Plenum.

Numerous papers have suggested that cyclodextrins can be used to nasally deliver insulin. Irie et al.[150] tested a number of cyclodextrins to determine their ability to promote insulin absorption in rats. Table 6 shows the effects of the various cyclodextrins on percent bioavailability of insulin in rats. From this data, DM-β-CD, DM-α-CD, and α-CD were superior to HP-β-CD. On the basis of spectrophotometric measurements the cyclodextrins were found to have little apparent direct interaction with insulin; however, they were able to inhibit insulin metabolism by nasal mucosa homogenates. Both the increased membrane permeability effects of DM-β-CD and its ability to protect insulin from metabolism contributed to its improved delivery. Results similar to those described by Irie et al. were observed by Watanabe et al.[201]

Large interspecies differences in insulin delivery were observed by Schipper et al.[202] and Merkus et al.[203,204] For example, in a communication, Merkus et al.[204] state that with 5% DM-β-CD the bioavailability of insulin was 108% in rats; however, in rabbits and human volunteers no insulin absorption could be measured. The rabbit study was subsequently published in greater detail and showed that when administered as a solution the insulin product containing DM-β-CD did indeed show poor insulin availability. Insulin bioavailability was quite substantial (12.9%), however, when a DM-β-CD complex containing insulin was administered as a powder.[205] Figure 17 is a plot of serum insulin as a function of time, which clearly shows the difference in the effects of the solution versus the powder. Like others, Schipper et al.[206]

performed studies showing the effects of DM-β-CD on nasal ciliary function and concluded that the effects, although significant, were transitory. Many of these results from this research group with insulin and other peptides were summarized in a paper by Coos Verhoef et al.[207]

The effects of cyclodextrins on the nasal delivery of other proteins have received some attention. Watanabe et al.[208,209] studied the effect of α-, β-, and γ-CDs on the nasal delivery of recombinant human granulocycte colony-stimulating factor (rhG-CSF). Better results were seen with α- and β-CDs than with γ-CD. Surprisingly, rhG-CSF was reasonably well absorbed without the cyclodextrins and, although DM-β-CD enhanced delivery, the effect was quite small. Agerholm et al.[210] studied the effect of didecanoyl-L-α-phosphatidylcholine (DDPC) and α-CD on the epithelial transport and intranasal bioavailability in rabbits of human growth hormone (hGH). The nasal formulation was a powder that consisted of a combination of DDPC and α-CD along with the drug and other inert ingredients. The absolute bioavailability of hGH from nasal delivery compared to an iv dose was 23 $\pm$ 2.4%. However, from light and electron microscopy studies as well as immunostaining experiments which attempted to localize the hGH, the authors concluded that "the principal transport route of hGH formulated with absorption enhancers DDPC and α-CD was transcellular through ciliated cells showing distinct signs of early necrosis". The damage appeared to be specific to ciliated cells. Improved paracellular transport did not seem to contribute to the enhancement. The authors recommended that the effects were significant and that chronic therapy via nasal delivery was unacceptable. Igawa et al.[211] showed that α-CD could affect the nasal delivery of human fibroblast interferon-β (HuIFN-β).

The effects of various cyclodextrins in promoting the nasal delivery of leuprolide and buserelin, two peptide LHRH agonists, have received some attention. Adjei et al.[212] showed that α-CD promoted the nasal delivery of leuprolide in rats and human subjects. As in the work of Merkus et al., nasal delivery in rats was greater than in humans. A high degree of subject to subject variation (rats and humans) was seen in the data. Shimamoto[213] showed similar differences between rat, dog, and human data. Leuprolide bioavailability from a 5% α-CD solution was 69.3% in rats and 87.4% in dogs but only 5.6% in humans. The general conclusion was that drainage from the nasal cavity may be higher in humans, thereby not allowing sufficient time for delivery to be effected. Matsubara et al.[198] showed good bioavailability for buserelin from a DM-β-CD solution. Although HP-β-CD did not enhance the nasal delivery of buserelin (11.9 $\pm$ 2.0%, control, versus 9.5 $\pm$ 2.4% with 10% HP-β-CD), Abe et al.[214] showed that a combination of an absorption enhancer, 1-[2-(decylthio)ethyl]-azopentan-2-one (HPE-101), with HP-β-CD did have a dramatic effect. HPE-101 alone did not enhance delivery but was substantially solubilized by HP-β-CD. In the presence of excess HP-β-CD (>10%) the synergistic effect with HPE-101 diminished, probably because the level of free, noncomplexed HPE-101 decreased with increasing HP-β-CD. The authors claim that local mucosal damage was minimal.

Schipper et al.[215] studied the nasal administration of an ACTH (4–9) peptide analog with DM-β-CD in rats and rabbits. As in earlier studies, DM-β-CD enhanced the delivery of this peptide and the effect was more pronounced in rats (13 $\pm$ 4% without and 65 $\pm$ 21% with DM-β-CD) than in rabbits (10 $\pm$ 6% versus 17 $\pm$ 8%). α-CD was also effective in promoting nasal delivery. The authors observed that DM-β-CD had its greatest effect when administered simultaneously with the analog, but pretreatment with the cyclodextrin 1 h before administration still showed a considerable enhancement, suggesting that the effect of DM-β-CD on the nasal epithelial cells was not immediately reversible. Interestingly, the analog was effective in helping the functional recovery of peripheral nerve damage in rats after sc administration but ineffective after nasal and iv administration.

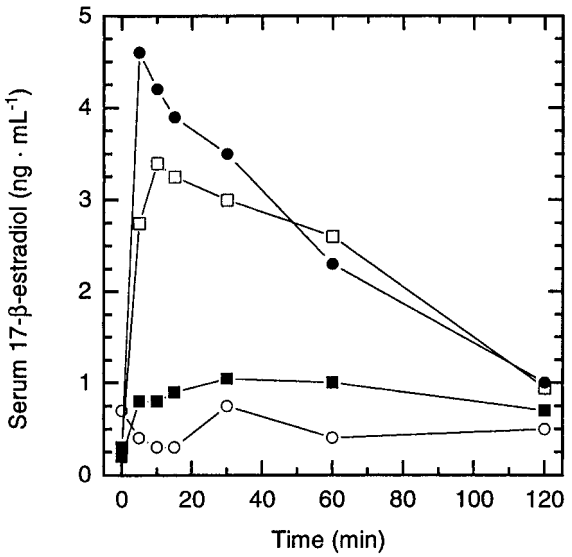


Figure 18—Serum 17β-estradiol (100 μg) in ng·mL^{-1} versus time in rabbits after administration of iv 17β-estradiol and DM-β-CD (●), nasal 17β-estradiol and DM-β-CD (□), nasal 17β-estradiol suspension (■), and nasal placebo (○). Reprinted with permission from ref 89. Copyright 1990 Plenum.

Nasal Delivery of Poorly Soluble Drugs—Nasal delivery of poorly water soluble, lipophilic drugs can be limited by their solubility. Like ophthalmic administration, solubilization of these drugs by cyclodextrins can dramatically improve their systemic delivery via nasal administration by presenting higher overall concentrations to the nasal epithelium. This is especially the case for those drugs that also undergo extensive presystemic metabolism if administered orally. Drugs absorbed via the nasal epithelium enter systemic circulation directly thus avoiding presystemic metabolism by enzymes present in GI lumen, the enterocytes, and the liver. Classic examples of this are progesterone and 17β-estradiol, both of which undergo erratic and extensive metabolism when administered orally.

The nasal delivery of 17β-estradiol in the presence of DM-β-CD was demonstrated by Hermens et al.[89] They compared the 17β-estradiol serum levels obtained after iv administration in rabbits and rats of 17β-estradiol in a DM-β-CD aqueous solution to nasal delivery via a suspension and a DM-β-CD solution. The authors determined the effect of DM-β-CD on ciliary beat frequency in human adenoid tissue as a measure of DM-β-CD toxicity. The concentration of 17β-estradiol used in the studies varied from 0.5 to 2 mg·mL^{-1} with a 17β-estradiol/DM-β-CD mole ratio of 1:2. Therefore, the DM-β-CD concentrations used were approximately 0.5–2% (w/v). This same group also studied the delivery of progesterone and the combination of estradiol and progesterone in rats and in oophorectomized women.[216,217] In both cases, DM-β-CD was used as a solubilizer and enhancer for nasal delivery. In the rabbit and rat studies, an iv dose of the drug was used as a control so that absolute bioavailability of the drugs could be determined. Figures 18 and 19 show the serum concentration versus time curves for iv and nasal 17β-estradiol and progesterone in rabbits and rats, respectively. The absolute bioavailability of 17β-estradiol from nasal administration with DM-β-CD in rats was 67% when administered alone and 59% when administered with progesterone. The absolute bioavailability of progesterone from nasal administration with DM-β-CD in rats was 58% when administered alone and 67% when administered with 17β-estradiol. In rabbits, progesterone

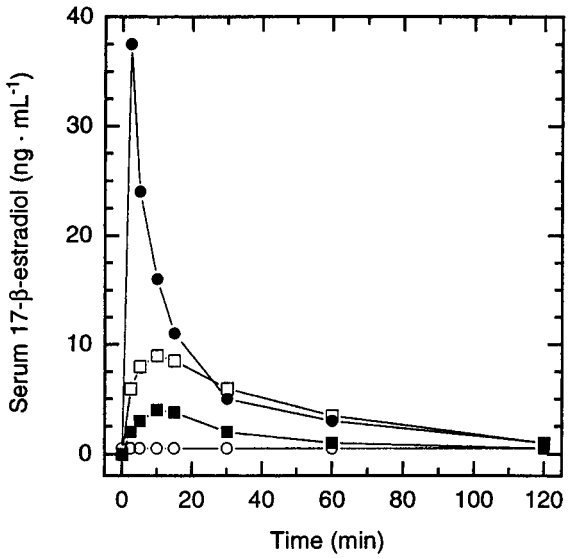


Figure 19—Plot of serum 17β-estradiol (10 μg) in ng·mL^{-1} versus time in rats after administration of iv 17β-estradiol and DM-β-CD (●), nasal 17β-estradiol and DM-β-CD (□), nasal 17β-estradiol suspension (■), and nasal placebo (○). Reprinted with permission from ref 89. Copyright 1990 Plenum.

exhibited an absolute bioavailability of 58% when DM-β-CD was used but only 18% when a progesterone suspension without DM-β-CD was used. The pilot study in women on the combination product showed rapid elevation of serum levels of estradiol, estrone, and progesterone with no adverse side effects being noted.[217] Another study in rabbits which utilized progesterone with β-CD as the complexing agent proved less conclusive.[218]

Al-Nakib et al.[219] studied the suppression of colds after nasal spray administration of a new drug, R61837, to rhinovirus challenged human volunteers. R61837 was formulated as a 2.5 mg·mL^{-1} solution with 10% HP-β-CD. A 10% HP-β-CD solution was used as a placebo control. Presumably, a HP-β-CD solution was used to nasally delivery R61837 as the spray because other formulations proved less desirable. R61837 was found to be effective in this dosage form. Recently, Helm et al.[220] published a study on the effect of HP-β-CD on the solubility and stability of dihydroergotamine mesylate to evaluate a possible nasal spray dosage form. The solubility of dihydroergotamine was significantly enhanced by HP-β-CD, but its chemical stability was only marginally affected.

In summary, the nasal delivery of drugs seems to be substantially enhanced by a number of cyclodextrins, especially those that seem to have a membrane-disturbing effect. Most of these cyclodextrins are also those which have been shown to be systemically toxic and to cause red blood cell hemolysis. Chronic safety studies would be necessary to confirm that there is no long-term damage to the nasal epithelium and that systemic delivery of low levels of the cyclodextrins proves safe because if drug absorption is enhanced so must the delivery of the cyclodextrin be enhanced. Use of non-membrane-disturbing cyclodextrins to enhance drug solubility to effect nasal delivery or the use of the same cyclodextrins to enable formulation of a drug could prove useful and less likely to raise safety concerns. These cyclodextrins are not likely to enhance the delivery of drugs whose inability to be delivered is due to poor membrane permeability.

Dermal, Rectal, and Pulmonary Applications of Cyclodextrins

Introduction—The major uses of cyclodextrins in dermal, rectal, and pulmonary drug delivery are to improve either the local action of the drug through increased solubility or stability of the drug or the systemic delivery of the drug through a potential combination of these factors with a cyclodextrin-associated increase in membrane permeability. As discussed in the nasal applications section, the use of cyclodextrins as permeation enhancers should be approached cautiously due to the permanent damage that may be caused and the potential for pathogenic substances and the cyclodextrins themselves to be systemically absorbed.

Summary of Reviews to Date—As noted earlier, Uekama et al. have recently published a general review[25] on applications of cyclodextrins, including a brief overview of the rectal absorption of the cyclodextrins themselves as well as rectal and dermal applications of cyclodextrins. The rectal absorption of the cyclodextrins is significant. As noted by Uekama et al., Arima et al.[221] found that greater than 20% of an administered dose of HP-β-CD was excreted in the urine of rats following rectal administration of suppositories containing either the cyclodextrin by itself or in combination with 4-biphenylacetate. Similar behavior was noted for administration of DM-β-CD with drug. The observed systemic absorption of the cyclodextrin itself raises questions concerning the systemic safety of the particular cyclodextrin derivative. With regard to enhancement of rectal absorption, Uekama et al.[25] reviewed the use of β-CD, HP-β-CD, and DM-β-CD in suppository bases. The authors suggest that HP-β-CD may be useful for improving the rectal absorption of poorly soluble drugs compared to β-CD and DM-β-CD due to its ability to decrease local irritation of some drugs.

Uekama et al.[25] also reviewed dermal applications of cyclodextrins. In this review the authors discuss the necessity to choose a suitable vehicle to elicit the desired cyclodextrin effect, i.e., enhancement or retardation of drug release. Of particular interest is a study presented involving the application of HP-β-CD as an aqueous solution to the skin of rats under nonocclusive conditions.[222] In these studies, HP-β-CD was found to distribute homogeneously throughout the epidermis and dermis, suggesting that HP-β-CD may affect transfer of drug through the skin by complex absorption. Additionally, examples of the use of β-CD, HP-β-CD, DM-β-CD, and CME-β-CD in dermal formulations are presented.

Dermal Applications—As previously discussed, cyclodextrins may improve the efficacy of dermally applied drugs through an increase in the apparent solubility or stability of the drug along with a potential for cyclodextrin-associated increases in membrane permeability. In addition, the formulator must also consider the potential of cyclodextrins to inhibit the absorption of drugs as a result of highly stable inclusion complexes. Many recent publications have addressed the use of cyclodextrins for dermal application of drugs in animal models and humans. Most of these studies are conducted *in vitro* using isolated skin and diffusion cells; however, several studies have investigated the systemic levels of drug following dermal application of a formulation.

An example of the effect of the stability constant of a cyclodextrin drug complex and cyclodextrin-associated permeation enhancement is evident in recent studies of Vollmer et al.,[223,224] who investigated the effect of HP-β-CD and DM-β-CD on the *in vivo* transdermal delivery in rats of liarozole, a cytochrome P450 inhibitor. Additionally, the authors investigated the mode of penetration enhancement using differential scanning calorimetry (DSC) by studying the thermal profiles of human stratum corneum following treatment with the respective cyclodextrins. Experimentally, the transdermal delivery of liarozole in anesthetized rats was evaluated by cannulating the iliolumbal skin vein draining the skin area used in the study and continuously collecting blood over the duration of the experiment. Drug formulations were applied to the skin in a closed Teflon chamber which

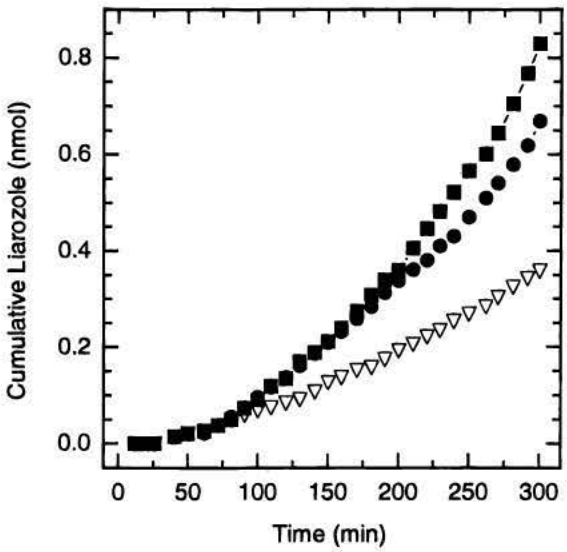


Figure 20—Comparison of the cumulative absorbed amounts of liarozole in blood following percutaneous absorption in rats. Values are the means of six experiments. Key: (●) water (pH 4), (■) 20% HP-β-CD (pH 7), (▽) 20% DM-β-CD (pH 7). Reprinted with permission from ref 223. Copyright 1994 Royal Pharmaceutical Society of Great Britain.

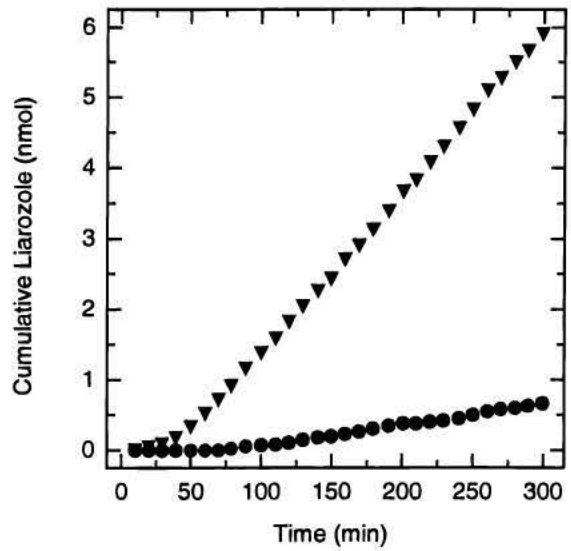


Figure 21—Comparison of the cumulative absorbed amounts of liarozole in blood following percutaneous absorption in rats. Values are the means of six experiments. Key: (●) water (pH 4), (▼) pretreatment with 20% DM-β-CD. Reprinted with permission from ref 223. Copyright 1994 Royal Pharmaceutical Society of Great Britain.

was glued to the skin. The liarozole was applied as a 1% solution in the absence (pH 4) and presence of 20% cyclodextrin. In associated studies, DM-β-CD was also used to pretreat the application area for 4 h prior to the application of the 1% solution in order to investigate the effect of DM-β-CD on skin permeability.

As can be seen in Figure 20, the liarozole solution containing HP-β-CD increased the absorption of drug slightly over a 5 h period relative to the pH 4 liarozole solution while the liarozole solution containing DM-β-CD significantly decreased the absorption of the drug (a 40% decrease in flux). From unpublished results, Vollmer et al. present the 1:1 stability constants of liarozole to be 8900 and 10 152 M^{-1} for HP-β-CD and DM-β-CD, respectively. Vollmer et al. suggest that the decrease in absorption observed with DM-β-CD is a result of the higher binding constant and the subsequent binding competition between DM-β-CD and the skin for liarozole. This competition is supported by the DM-β-CD pretreatment studies, which caused a 10-fold increase in drug flux, suggesting that DM-β-CD in the absence of liarozole increases the permeability of the skin (Figure 21). This finding is also supported by the DSC studies on human stratum corneum which was soaked in 20% solutions of the respective cyclodextrin derivatives for 4 h prior to analysis. The DSC endothermic transitions for the HP-β-CD treated sample were nearly identical to that of the control while those of the DM-β-CD treated sample were altered or absent.

On the basis of previous literature, Vollmer et al. attribute the observed endothermic changes associated with DM-β-CD to interactions with proteins and displacement of fatty acids and cholesterol with subsequent disordering of the bilayer structure of the stratum corneum. Therefore, Vollmer et al. suggest that HP-β-CD enhances transdermal absorption of liarozole by influencing the distribution and partitioning of the drug while DM-β-CD enhances absorption by changing the structure of the stratum corneum.

The Vollmer studies[223,224] demonstrate the need to understand the mechanism by which the presence of cyclodextrins in a topical formulation may increase the local or systemic action of a drug. If the presence of a given cyclodextrin causes changes in the structure of the stratum corneum, the formulator must consider the long-term effects of chronic use of such a vehicle. For example, Loftsson et al.[225] recently reported on the use of HP-β-CD, γ-CD, HP-γ-CD, and RM-β-CD for solubilization and subsequent topical delivery of acitretin, a retinoid antipsoriasis drug, through hairless mouse skin *in vitro*. Acitretin is reported to be insoluble in water, and the use of cyclodextrins (12.5–50% w/v) in combination with varying pH resulted in observed aqueous acitretin concentrations from less than 0.01 $mg \cdot mL^{-1}$ up to 3.35 $mg \cdot mL^{-1}$ at room temperature. Over the range of pH and cyclodextrin concentrations studied, the RM-β-CD provided the highest acitretin concentrations and was selected for permeation studies. The addition of RM-β-CD to a control propylene glycol vehicle did not increase the flux of the drug through hairless mouse skin from the control level of 0.03 $\mu g \cdot h^{-1} \cdot cm^{-1}$. Replacement of one-half of the propylene glycol in the vehicle with water, however, resulted in a 3–4-fold increase in flux. While these results demonstrate that it is possible to increase transdermal acitretin delivery through the use of RM-β-CD, it is unclear whether this increase is a result of increased acitretin solubility solely or a combination of increased solubility and membrane permeability due to removal of membrane components by the methylated cyclodextrins.[226,227]

Other recent studies on the use of cyclodextrins in topical formulations include a report by Loftsson and Sigurdardóttir[228] on the effect of poly(vinylpyrrolidone) (PVP) and HMPC in the presence of HP-β-CD on the flux of hydrocortisone through hairless mouse skin *in vitro*. In these studies, 1.6% (w/v) suspensions or solutions of hydrocortisone in aqueous HP-β-CD solution (5–25%) in the presence and absence of 0.25% PVP or 0.20% HMPC were equilibrated for 3 days in the donor chamber of Franz diffusion cells holding hairless mouse skin. The authors found that the addition of PVP or HMPC increased the apparent aqueous solubility of hydrocortisone in the presence of HP-β-CD. The flux of hydrocortisone from suspensions increased with increasing HP-β-CD concentration (up to approximately 10–13% HP-β-CD) as a result of the increased apparent solubility of the drug. However, as can be seen in Figure 22, when all the hydrocortisone was initially in solution, additional increases in HP-β-CD concentration decreased the observed flux of the drug as a result of competition between the cyclodextrin and the skin and the lack of absorption of the cyclodextrin complex of hydrocortisone. The addition of PVP or HMPC generally

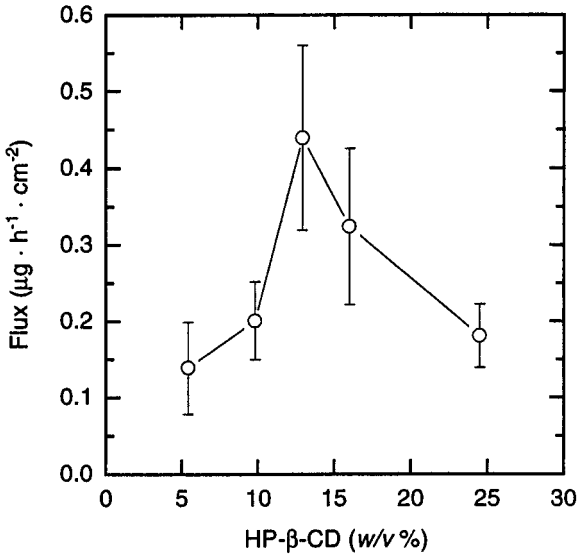


Figure 22—Effect of HP-β-CD concentration on the flux of hydrocortisone through hairless mouse skin *in vitro*. Reprinted with permission from ref 228. Copyright 1994 Elsevier.

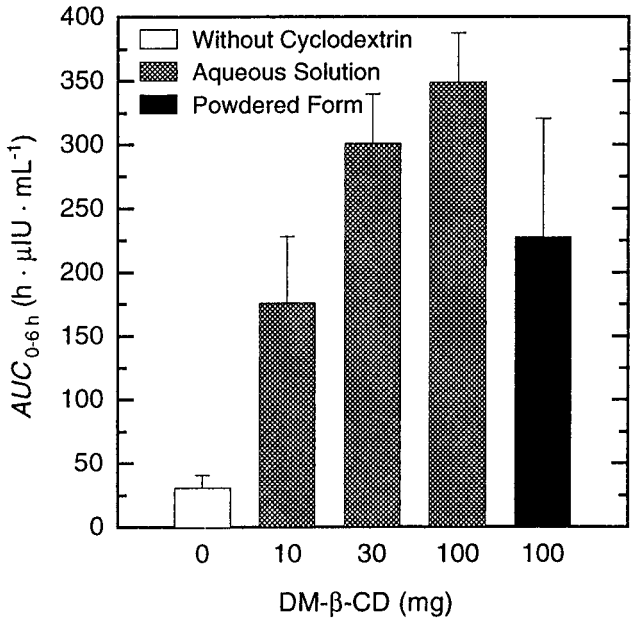


Figure 23—Mean AUC of insulin following rectal administration of hollow-type suppositories containing insulin and various amounts of DM-β-CD in citrate buffer solution (pH 3.0) or powdered form in rabbits. Reprinted wi h permission from ref 236. Copyright 1992 Japan Publ (Tokyo).

increased drug flux when the drug was present as a suspension (low HP-β-CD concentrations). These results are in agreement with related studies by Loftsson et al.[229]

Other recent studies in topical applications of cyclodextrins include reports by Ammar and El-Nahhas[230] on the use of β-CD in transdermal diffusion studies of bromhexine hydrochloride using rat skin *in vitro*, Preiss et al.[231] on the use of β-CD and HP-β-CD for modified penetration of hydrocortisone into excised human skin, and Amdidouche et al.[232] on the use of laser Doppler velocimetry in the evaluation of tretinoin-induced skin irritation attenuation in humans by β-CD complexation. Recently the use of β-CD in topical formulations was reported by Singh et al.[140] for the *in vivo* application of hamycin, Rahman et al.[233] for the *in vitro* application of chloramphenicol, and Reddy et al.[139] in the *in vivo* evaluation of piroxicam formulations. The use of DE-β-CD for studies on the release and *in vivo* absorption in hamsters of topical indomethacin ointments was reported by Kawahara et al.[234] while Umemura et al.[235] studied the percutaneous absorption of nitroglycerin in hamsters from ointments containing DE-β-CD.

Rectal Applications—The use of cyclodextrins to enhance rectal absorption of drugs should be approached with an understanding of the systemic safety of the cyclodextrin since, as previously discussed, several of the cyclodextrin derivatives have been found to be well absorbed rectally.[221] Recent reports on the use of cyclodextrins for rectal applications include studies aimed at improving the efficacy of conventional small molecules and biological drug agents.

Watanabe et al.[236] recently reported on the enhanced rectal absorption in rabbits of insulin from hollow-type suppositories containing insulin and cyclodextrin combinations. Hollow-type suppositories consist of an oleaginous base with a hollow core which contains the drug or drug and cyclodextrin combination in powdered or solution form. The advantage of such suppositories over traditional dispersions of drug in an oleaginous base is protection of heat labile drugs during the formation of the suppository since the drug is added after the suppository is formed. In these studies three types of suppositories were prepared. The first type contained approximately 26 IU·mg^{-1} of insulin and various amounts of either DM-β-CD, HP-β-CD, α-CD, β-CD, or γ-CD in pH 3 citric acid solution or as a powder. The second type contained the cyclodextrin without insulin and the third type contained insulin, without cyclodextrin. These three types of suppositories were used in various combinations to determine the effect of cyclodextrin on rectal insulin absorption. Coadministration of insulin and cyclodextrin was accomplished by administration of the first type of suppository while the effect of preadministration of cyclodextrin on insulin absorption was determined by administering the second type of suppository containing only cyclodextrin at 0.5, 6, 24, or 48 h prior to administration of the third type of suppository containing only insulin. Plasma insulin and glucose levels were followed from 0 to 6 h following administration using a commercial enzyme immunoassay kit and commercial glucose test kit.

The authors found that administration of the third type of suppository containing only insulin did not change plasma insulin or glucose levels relative to endogenous levels observed after administration of a blank suppository. However, plasma insulin levels were increased following administration of the first type of suppository containing insulin and cyclodextrin (30 mg) in citrate buffered solution as can be seen in Figure 23. The insulin AUC values were elevated 3–4 times over control levels for the natural cyclodextrins, 5 times for HP-β-CD, and almost 8 times for DM-β-CD. Decreases in plasma glucose generally followed this trend. Further evaluation of the DM-β-CD and insulin concentration dependence demonstrated that the insulin AUC increased with increasing DM-β-CD concentration between 10 and 30 mg but not significantly between 30 and 100 mg of DM-β-CD while AUC values did not significantly increase with increasing insulin in the suppository (5.2 IU vs 26 IU) with 30 mg of DM-β-CD. Suppositories containing powdered insulin (26 IU) and DM-β-CD (100 mg) also increased plasma insulin levels (6 times control) but not to the extent of the solution containing equal amounts of each agent (9 times control). Application of suppositories containing only powdered insulin did not increase plasma insulin levels.

To determine the duration of the permeation enhancement of insulin by DM-β-CD, rabbits were administered suppositories containing DM-β-CD prior to those containing insulin. As can be seen in Table 7, a significant decrease in plasma insulin was observed with increasing DM-β-CD pretreatment time relative to the coadministration of the agents

Table 7—Effect of DM-β-CD Pretreatment on the Pharmacokinetic Parameters of Rectal Insulin Administration[a] [236]

Preadministration Time (h)[b]	$AUC_{0-6\ h}$ (h·μIU·mL)	C_{max} (μIU·mL^{-1})
0[c]	290 ± 42	556 ± 233
0.5	188 ± 34	174 ± 58
6	116 ± 44[d]	96 ± 44[e]
24	70 ± 21[d,f]	27 ± 4[d]
48	33 ± 9[d,f]	30 ± 10[d]

[a] 26 IU of insulin, 30 mg of DM-β-CD, $n = 3$ or 4. [b] Interval between DM-β-CD and insulin administration. [c] Simultaneous administration of DM-β-CD and insulin. [d] $p < 0.01$ versus 0 h. [e] $p < 0.05$ versus 0 h. [f] $p < 0.05$ versus 0.5 h.

with values obtained at 24 and 48 h pretreatment being indistinguishable from the control. In addition, no decrease in plasma glucose was observed following administration of the insulin suppository in the 24 and 48 h pretreatment studies. The authors attribute the observed increases in rectal insulin absorption to alteration of the lipid barrier with a minor contribution from inhibition of proteolytic activity by the cyclodextrins. The proposed mechanism of permeation enhancement is supported by a lack of observed interaction (increased chemical activity) between insulin and the cyclodextrins in this study as well as recent studies by Chen and Chien[200] which demonstrated that HP-β-CD (10%) reduced the degradation rate constant of a protein, methionine enkephalin, in rectal extracts by approximately 3-fold.

Uekama et al.[237] also reported recently on the use of hollow-type suppositories employing α-CD and polysaccharides to modify the rectal absorption of morphine in rabbits. Generally, α-CD and β-CD were found to increase the rate and extent of morphine absorption while γ-CD decreased rectal absorption of morphine. A combination of α-CD and xanthan gum produced elevated and sustained plasma morphine levels resulting in AUC values which were approximately 4 times that of morphine alone. The addition of the xanthan gum was found to prevent the upward movement of the drug in the colon, thereby limiting absorption of morphine from areas which are impacted by first-pass metabolism.

Other recent publications on the rectal application of cyclodextrins include the use of HP-β-CD and γ-CD in suppository bases containing diazepam for application in human volunteers. In these studies, Frijlink et al.[238] found that diazepam absorption was increased with both cyclodextrins and that the increases generally correlated with *in vitro* release studies. Yanagi et al.[239] investigated the use of decanoic acid and α-CD as an additive in sodium cefmetazole suppositories in rabbits and observed significant increases in plasma levels of drug (AUC values 22 times higher than in the absence of cyclodextrin and decanoic acid). Frijlink et al.[240] also studied the effect of β-CD on the rectal absorption from enemas in healthy human volunteers of either diazepam or naproxen. The authors observed faster absorption from the solutions containing cyclodextrin compared to the drugs as suspensions.

Pulmonary Applications—Publication of studies involving the pulmonary applications of cyclodextrins is limited in the recent literature to studies on the absorption in rabbits of several cyclodextrin derivatives themselves[241] and the use of HP-β-CD in an attempt to modify the pulmonary transport of small, hydrophobic molecules.[242,243]

With regard to the pulmonary absorption of the cyclodextrins themselves, Cabral Marques et al.[241] studied the absorption and pharmacokinetics of β-CD, DM-β-CD, and HP-β-CD following intratracheal (i.t.) instillation in rabbits. In these randomized crossover studies in five rabbits, DM-β-CD and HP-β-CD were administered iv and i.t. as solutions (18 mg·mL^{-1}) in isotonic saline at 10 mg·mL^{-1} while β-CD was

Table 8—Summary of Pharmacokinetic Parameters (Mean ± SE; $n = 5$) of β-, DM-β-, and HP-β-CD after I.t. Administration in Rabbits[241]

	β-CD	DM-β-CD	HP-β-CD
t_{max} (min)	30.0 ± 4.5	22.4 ± 1.9	113.0 ± 10.06
C_{max} (μg·mL^{-1})	23.5 ± 1.9	26.8 ± 5.7	14.4 ± 2.4
$t_{1/2}$ (min)	44.2 ± 3.9	44.3 ± 6.8	63.0 ± 4.8
MAT[a] (min)	26.1 ± 6.8	20.7 ± 4.0	113.0 ± 6.6
F (%)	65.9 ± 12.8	73.9 ± 13.2	79.8 ± 12.0

[a] MAT: mean absorption time.

administered as a solution iv (18 mg·mL^{-1}) and a suspension i.t. (18.5 mg·mL^{-1}) at 10 mg·kg^{-1}. The iv studies allowed for determination of the absolute bioavailability following pulmonary administration. Following dosing of the animals, blood samples from the marginal ear vein were collected and plasma was isolated for determination of cyclodextrin concentration by either ^{14}C scintillation counting (DM-β-CD and HP-β-CD) or negative colorimetric detection following HPLC fractionation (β-CD).

Interestingly, the authors found that the absolute bioavailabilities following pulmonary administration were 65.9 ± 12.8% for β-CD, 73.9 ± 13.2% for DM-β-CD, and 79.8 ± 12.0% for HP-β-CD (Table 8, F%). These values are considerably higher than cyclodextrin absorption following other nonparenteral routes and should limit the future choice of cyclodextrins considered for pulmonary administration to those with acceptable systemic safety profiles or negligible pulmonary absorption. The time to reach maximum plasma concentration was 20−30 min for β-CD and DM-β-CD while the time for HP-β-CD was approximately 113 min. The plasma elimination half-lives of the three cyclodextrins following pulmonary absorption were comparable to those following iv administration, suggesting a common elimination route independent of administration route. For reference, the systemic clearance of all three cyclodextrins approximated the glomerular filtration rate and the steady-state volume of distribution approximated the extracellular fluid volume.

In studies involving the modification of pulmonary absorption of small, hydrophobic molecules, Wall et al.[243] investigated the use of HP-β-CD for slowing the rate of absorption of rolipram and testosterone in the rat. The authors hypothesized that inclusion of a molecule with a carrier molecule which is also absorbed would present a larger entity for absorption and decrease the apparent rate of drug absorption. This hypothesis was based on earlier work of Cabral Marques et al.,[242] who used an HP-β-CD complex of salbutamol in an attempt to slow the pulmonary absorption of this drug in rabbits. The pulmonary absorption of salbutamol was not significantly extended, however, through use of HP-β-CD complexation. Cabral Marques et al.[242] suggested that drugs which exhibited larger stability constants with HP-β-CD than salbutamol (60−70 M^{-1})[244] may display extended absorption profiles. Therefore, Wall et al. studied rolipram and testosterone, which have stability constants of 260 and 12 000 M^{-1}, respectively. The authors found that HP-β-CD had little affect on rolipram absorption and no effect on testosterone absorption *in vivo*. The authors suggested that rapid dissociation of the drugs from HP-β-CD may be occurring *in vivo* due to potential competition of these drugs for the cyclodextrin by endogenous molecules such as cholesterol.

Summary

Although enthusiasm for the use of cyclodextrins as enabling excipients has gone through various phases, the advent of newer cyclodextrin derivatives with better safety records

has resulted in renewed interest of late. Modern drug discovery processes appear to have resulted in a higher incidence of larger, more complex and often more insoluble and unstable molecules. The tools available to formulation scientists to solubilize and stabilize these agents are somewhat limited, especially those tools that can be used in invasive dosage forms such as parenteral formulations. We hope that this Review has brought to light the more recent findings on the use of cyclodextrins in helping to overcome some of these problems. The purpose of this Review, one in a series of three Reviews, was to promote interest in the use of modified cyclodextrins as novel and imaginative tools for effecting efficient drug delivery. We hope we have done our part toward achieving this goal.

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