JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 1119–1135

Received December 15, 2010
Accepted March 1, 2011
DOI
10.1111/j.2042-7158.2011.01279.x
ISSN 0022-3573

Review

Pharmaceutical applications of cyclodextrins: effects on drug permeation through biological membranes

Thorsteinn Loftsson[a] and Marcus E. Brewster[b]

[a]Faculty of Pharmaceutical Sciences, University of Iceland, Hofsvallagata, Reykjavik, Iceland and [b]Pharmaceutical Development and Manufacturing Sciences, Janssen Research and Development, Johnson & Johnson, Scheperstraat, Beerse, Belgium

Abstract

Objectives Cyclodextrins are useful solubilizing excipients that have gained currency in the formulator's armamentarium based on their ability to temporarily camouflage undesirable physicochemical properties. In this context cyclodextrins can increase oral bioavailability, stabilize compounds to chemical and enzymatic degradation and can affect permeability through biological membranes under certain circumstances. This latter property is examined herein as a function of the published literature as well as work completed in our laboratories.

Key findings Cyclodextrins can increase the uptake of drugs through biological barriers if the limiting barrier component is the unstirred water layer (UWL) that exists between the membrane and bulk water. This means that cyclodextrins are most useful when they interact with lipophiles in systems where such an UWL is present and contributes significantly to the barrier properties of the membrane. Furthermore, these principles are used to direct the optimal formulation of drugs in cyclodextrins. A second related critical success factor in the formulation of cyclodextrin-based drug product is an understanding of the kinetics and thermodynamics of complexation and the need to optimize the cyclodextrin amount and drug-to-cyclodextrin ratios. Drug formulations, especially those targeting compartments associated with limited dissolution (i.e. the eye, subcutaneous space, etc.), should be carefully designed such that the thermodynamic activity of the drug in the formulation is optimal meaning that there is sufficient cyclodextrin to solubilize the drug but not more than that. Increasing the cyclodextrin concentration decreases the formulation 'push' and may reduce the bioavailability of the system.

Conclusions A mechanism-based understanding of cyclodextrin complexation is essential for the appropriate formulation of contemporary drug candidates.

Keywords absorption, complex, cyclodextrin, membrane, permeation

Introduction

Aqueous solubility and the ability of solutes to permeate biological membranes are the main physicochemical properties that determine the 'drugability' of a new chemical entity (NCE). While 40% of currently marketed drugs are poorly soluble based on the Biopharmaceutical Classification System (BCS), about 90% of NCEs may be characterized in this way. Poor aqueous solubility is an important factor associated with poor oral bioavailability. Poor aqueous solubility can also hamper delivery via non-oral routes such as those related to buccal, ocular, nasal, pulmonary, rectal and vaginal administration. Only the dissolved drug molecules are able to penetrate biological membranes such as the mucosa. Numerous methods have been proposed for enhancing aqueous solubility of poorly soluble drugs and NCEs, including both chemical methods such as prodrugs and physical methods such as production of higher energy polymorphs and the formation of water-soluble complexes. One beneficial solubilizing technique involves the use of water-soluble cyclodextrin (CD) complexes.[1] CD complexation of a poorly soluble lipophile will improve its aqueous solubility but the complex itself is, in general, unable to permeate biological membranes per se. Consequently, CDs can both enhance and hamper drug permeation through biological membranes. A good knowledge of how CDs affect drug permeation through membranes is a prerequisite for the successful application of CDs. Here we use well-established thermodynamic principles and mathematical models to explain how CDs enhance and hamper drug

Correspondence: Thorsteinn Loftsson, Faculty of Pharmaceutical Sciences, University of Iceland, Hofsvallagata 53, IS-107 Reykjavik, Iceland.
E-mail: thorstlo@hi.is

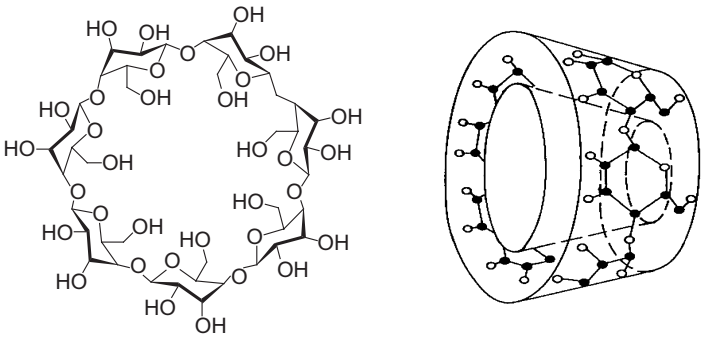
permeation through membranes. Our observations are based on a thoughtful review of the available literature on CDs and drug permeation.

Cyclodextrins

The physicochemical and biological properties of CDs and their pharmaceutical applications have recently been reviewed.[1] We present here a brief description of the main structural and physicochemical characteristics that are relevant to drug permeation through biological membranes. CDs are cyclic oligosaccharides formed by six (αCD), seven (βCD), eight (γCD) or more (α-1,4)-linked D-glucopyranose units (Table 1). Due to the chair structure of the glucopyranose units the molecules are cone-shaped with the secondary hydroxy groups extending from the wider edge and the primary hydroxyl groups from the narrow edge. This provides a CD molecule with a hydrophilic outer surface and somewhat lipophilic central cavity. Although the natural αCD, βCD and γCD and their complexes are hydrophilic, their solubility in aqueous solutions is somewhat limited, mainly due to the relatively high crystal lattice energy and intramolecule hydrogen bonding. Random substitution of the hydroxy groups, even by lipophilic groups, gives amorphous mixtures of water-soluble CD derivatives.[2,6] CD derivatives of pharmaceutical interest include the hydroxypropylated βCD and γCD (HPβCD and HPγCD), the randomly methylated βCD (RMβCD) and sulfobutyl ether βCD sodium salt (SBEβCD). As oligosaccharides, the natural αCD, βCD and γCD possess many of the same physicochemical and biological characteristics as the water-soluble linear dextrins. However, due to their cyclic nature, they are more resistant towards both enzymatic and non-enzymatic hydrolysis than the linear analogues. CDs are resistant to β-amylases that hydrolyse starch from the non-reducing end of the glucose polymer, but are slowly hydrolysed by α-amylases that hydrolyse starch from within the carbohydrate chain. The hydrolytic rate depends on the ring size and the fraction of free CD. CDs resist hydrolysis by obscuring all bridge oxygens within the central cavity and, thus, free CD is hydrolysed more rapidly than CD bound to a drug in a complex and the rate of hydrolysis increases with increasing cavity size.[7] For example, αCD and βCD are essentially stable towards α-amylase in saliva whereas γCD is rapidly digested by salivary and pancreatic α-amylase.[8,9] All the natural CDs and their above-mentioned derivatives are susceptible to bacterial digestion in the gastrointestinal tract.[4,5,10–14]

CDs are able to form inclusion complexes with many drugs by taking up lipophilic substructures of drug molecules into their central cavity. No covalent bonds are formed or broken during the complex formation and in aqueous solutions, drug molecules bound within the CD cavity are in dynamic equilibrium with free drug molecules in the solution. Complexes are continuously being formed and dissociated at rates close to the diffusion-controlled limit.[15] A 1 : 1 drug : CD complex, where one drug molecule forms a complex with one CD

Table 1 Structure of β-cyclodextrin and physiochemical characteristics of some cyclodextrins of pharmaceutical interest[2]



Properties	α-Cyclodextrin	β-Cyclodextrins			γ-Cyclodextrins	
	αCD	βCD	HPβCD	SBEβCD	RMβCD	γCD	HPγCD
Molar substitution	–	–	0.65	0.9	1.8	–	0.6
Molecular weight of anhydrous compound (Da)	972.8	1135	1400	2163	1312	1297	1576
Calculated $LogK_{(octanol/water)}$ at 25°C[a]	−13	−14	−11	<−10	−6	−17	−13
Solubility in water at 25°C (mg/ml)[b]	130	18.4	>600	>500	>600	249	>600
H donor	18	21	21	15	8	24	24
H acceptor	30	35	39	53	35	40	45
Approximate oral bioavailability in rats (%)[c]	2 to 3	~0.6	≤3	1.6	≤12	<0.1	<0.1

[a]From SciFinder, ACS, USA (scifinder.cas.org) and ChemExper Chemical Directory (http://www.chemexper.com). [b]From [3]. [c]% absorbed intact after oral administration to rats. From [4,5] and the cyclodextrin producers (i.e. Wacker Chemie AG (Germany) and CyDex Pharmaceuticals, Inc. (USA)).

molecule, is the most common form of CD complex in dilute aqueous solutions. The stability of a drug/CD complex is frequently assessed by using the equilibrium constant ($K_{1:1}$) of the 1 : 1 drug : CD complex in an aqueous complexation media:

$$\text{drug} + \text{CD} \underset{}{\overset{K_{1:1}}{\rightleftharpoons}} \text{drug/CD} \quad (1)$$

The value of $K_{1:1}$ is frequently between 10^1 and 10^3 M^{-1}. $K_{1:1}$ with greater value than 5×10^3 M^{-1} is rarely observed. There are, however, several exceptions. Sugammadex is a γCD derivative specifically designed to tightly bind the neuromuscular blocking agent rocuronium and some ozonide drug candidates have been shown to be tightly bound to SBEβCD.[16–18] The values of the equilibrium constants for these drug/CD complexes are greater than about 10^6 M^{-1} or large enough to affect the pharmacokinetics of the drugs after parenteral administration. It has been argued that the stability constants of drug/CD complexes must be greater than 10^5 M^{-1} to have a significant effect on the drug pharmacokinetics after parenteral administration.[11] The major driving force for drug release from the complexes is simple dilution although other mechanisms, such as direct drug partitioning from the complex to lipophilic membrane tissue, do contribute to rapid drug release from the complex.

The ability of a molecule to permeate lipophilic epithelia depends on its molecular weight, structure and physicochemical properties consistent with Fick's Law and the Stokes-Einstein equation. CDs permeate biological membranes via passive diffusion. Passive permeation through a lipophilic epithelium depends on the lipophilicity of the permeating molecule and the extent of absorption is frequently correlated to the partition coefficient between octanol and water. Lipinski's rule of five states that poor oral absorption or permeation are more likely when a molecule (1) has more than five hydrogen bond donors (expressed as the sum of OHs and NHs), (2) has more than more than 10 hydrogen bond acceptors (N or O atoms), (3) has octanol–water partition coefficient ($\text{LogK}_{(\text{octanol/water})}$) greater than 5, and/or (4) has molecular weight greater than 500 Da.[19,20] CDs violate three of these rules and although their $\text{LogK}_{(\text{octanol/water})}$ is less than 5 their hydrophilicity ($\text{LogK}_{(\text{octanol/water})}$ between −6 and −17) is high and, thus, they only permeate biological membranes with great difficulty (Table 1). The oral bioavailability of the hydrophilic CDs (i.e. αCD, βCD, CD, HPβCD, HPγCD and SBEβCD) is less than 3% and less than 12% for the more lipophilic RMβCD (Table 1). The oral bioavailability of HPβCD in humans is between 0.5 and 3.3% with 50–65% of the oral dose excreted unchanged in the faeces and the remainder mainly being metabolized by bacteria in the colon.[1] Thus, in general, CDs and drug/CD complexes do not permeate lipophilic membranes.

Cyclodextrins as absorptions enhancers

There are numerous publications on the effects of CDs on drug delivery through membranes and oral bioavailability. In most of these studies, CDs enhance drug delivery through the membranes; in some they have no effect and in a few CDs reduce or prevent drug permeation through the membranes. Some of these publications are provided in the accompanying Tables (Tables 2–5).

Dermal and transdermal drug delivery

A review of the studies listed in Table 2 reveals that, depending on the experimental conditions and vehicle composition, CDs can either increase or decrease drug permeation through skin. The main barrier to percutaneous absorption of hydrophilic CDs is the outermost layer of the skin (i.e. the *stratum corneum*). For example, only 0.02% of topically applied radiolabelled HPβCD was absorbed into intact hairless mouse skin under occlusive conditions during a 24-h period but in the same study about 24% of HPβCD was absorbed into stripped skin where *stratum corneum* had been removed.[49] Lipophilic CD derivatives, like the methylated CDs, are absorbed to a somewhat greater extent into skin but still the uptake is negligible or only 0.3% over 24 h for dimethyl-β-cyclodextrin (DMβCD).[63,64] Although CDs can, under certain conditions, extract lipophilic components from skin, pretreatment of skin with hydrophilic CDs does not, in general, enhance permeation.[25,27,33,52,65,66] Hydrophilic CDs reduce the drug release from water-in-oil (w/o) creams but enhance the release and drug permeation from oil-in-water (o/w) creams.[34,67] CDs are only able to enhance dermal and transdermal drug delivery from aqueous drug vehicles or through aqueous diffusion layers at the skin exterior.[68] Excess CD, more than is needed to solubilize a lipophilic drug in an aqueous vehicle, will reduce drug permeation into skin. Maximum enhancement is obtained when just enough CD is used to solubilize the lipophilic drug.[27–29,35,64,69] To this point, hydrophilic CDs have been added to sunscreen formulations to reduce absorption of lipophilic sunscreen agents into skin.[23,69–71]

Topical drug delivery to the eye

Examples of CD-containing ophthalmic formulations and topical drug delivery to the eye are listed in Table 3. The aqueous tear fluid and the mucus layer on the eye surface form an aqueous diffusion barrier for topical drug delivery to the eye. CDs can enhance delivery of lipophilic drugs through this barrier.[107,109,110] As in the case of dermal and transdermal drug delivery, excess CDs (i.e. more than is needed to solubilize the drug in the aqueous eye drop formulation) can result in decreased drug delivery into the eye.[72,75,96] Another important observation is that hydrophilic CDs, like HPβCD, do not enhance delivery of hydrophilic drugs into the eye after topical administration.[100,111] Furthermore, CDs are known to alleviate local drug irritation in the eye.[112–114]

Nasal, buccal, pulmonary, rectal and vaginal drug delivery

Some examples of reports on CD-containing formulations for nasal, buccal, pulmonary, rectal and vaginal drug delivery are shown in Table 4. The enzymatic activity in these mucosal membranes can be quite high and, thus, the observed permeation enhancement is sometimes due to enhanced drug stability through complexation, especially in the case of proteins

Table 2 Examples of cyclodextrin-containing dermal formulations and transdermal drug-delivery studies

Drug	Cyclodextrin	Reference
Acitretin	RMβCD	[21]
Alkannin	HPβCD	[22]
Avobenzone	HPβCD	[23]
Beclometasone dipropionate	γCD	[24]
4-Biphenylylacetic acid	βCD, DMβCD, HPβCD	[25,26]
Bupranolol	HPβCD, MβCD	[27]
Capsaicin	HPβCD	[28]
Celecoxib	DMβCD	[29]
Curcumin	HPβCD, HPγCD	[30]
Dexamethasone acetate	βCD, HPβCD	[31]
17β-Estradiol	HPβCD	[32]
Hydrocortisone	βCD, CMβCD, HPβCD, MLβCD, RMβCD	[32–39]
Ibuprofen	HPβCD	[40]
Indometacin	βCD, DEβCD, DMβCD	[41,42]
Ketoprofen	HPβCD	[43]
Liarozole	HPβCD	[44]
Lidocaine	DMβCD, HPβCD, SBEβCD	[45]
Loteprednol etabonate	DMβCD	[46]
Melatonin	HPβCD	[47]
Metopimazine	MβCD	[48]
Methyl paraben	HPβCD	[49]
Miconazole	αCD, HPβCD	[50]
Naproxen	βCD	[51]
Piribedil	RMβCD	[52]
Piroxicam	HPβCD	[53,54]
Prednisolone	βCD, γCD	[55]
Prostaglandin E1	αCD, βCD, CMEβCD,	[56–59]
Shikonin	HPβCD	[22]
Sulfanilic acid	βCD, DMβCD	[41]
Testosterone	HPβCD	[32]
Tolnaftate	βCD, βCD-polymer	[60]
Tretinoin	βCD, HPβCD, DMβCD	[61,62]
Triamcinolone	HPβCD	[33]

and peptides.[10] Methylated βCD, which is a somewhat lipophilic CD derivative, has been shown to act as a chemical penetration enhancer in nasal delivery, penetrating into the nasal epithelia and decreasing its barrier properties.[142,143] However, hydrophilic CDs do not readily permeate the nasal mucosa and excess CD will reduce drug absorption from the nasal cavity.[144,145] Hydrophilic CDs have been used successfully in a number of sublingual and buccal drug formulations, mainly to enhance drug dissolution in the saliva and to carry lipophilic drug molecules through the aqueous saliva diffusion barrier to the epithelial surface. CDs are more readily absorbed from the lungs than from other routes of delivery (oral, dermal, nasal, vaginal and rectal), which can impact their usage in pulmonary drug delivery; however, CDs that are considered to be safe for parenteral administration are also considered appropriate for pulmonary use.[10,146–148]

Oral drug delivery

A variety of examples of the usage of CDs in oral formulations are shown in Table 5. A more detailed analysis of the effects of CDs on oral drug bioavailability was performed by Carrier *et al*.,[206] who showed that better bioavailability was obtained from drug/CD complex than from physical mixtures of drugs and CDs, and that lipophilic drugs ($LogK_{(octanol/water)} > 2.5$) of low aqueous solubility (typically < 1 mg/ml) with moderate binding constants ($K < 5000\ M^{-1}$) were especially suitable for oral CD-containing formulations. For these applications, the drug should be moderately potent (dose < 100 mg) and the drug : CD ratio should preferably be 1 : 2 or greater. However, CDs can have other effects, such as drug stabilization, generation and stabilization of supersaturated drug solutions and inhibition of drug efflux, that are difficult to account for and, thus, these general guidelines do not apply to all reported studies (Table 5). Also, it has been shown that the effective thickness of the unstirred water layer (UWL) in the gastrointestinal tract decreases with increasing CD concentration, adding to the difficulties of creating general rules for the effect of CDs on drug bioavailability enhancement.[207] The UWL forms an aqueous drug diffusion barrier at the membrane surface. Other studies have related observed CD effects on oral drug bioavailability to the BCS and shown that while, in general, CDs have very little effect on bioavailability of BCS class I and III drugs, they can have significant effect on class II and IV drugs.[208,209] Furthermore, αCD (FBC_X tablets containing 1000 mg αCD; ArtJen, Canada) is used to complex triglycerides in the gastrointestinal tract and prevent their absorption. The fact that physical

Table 3 Examples of cyclodextrin-containing ophthalmic formulations and topical drug delivery to the eye

Drug	Cyclodextrin	Reference
Acetazolamide	HPβCD	[72–74]
Anandamides	HPβCD	[75,76]
Cannabinoids (various)	HPβCD	[77]
Ciclosporin	αCD	[78–80]
Dehydroepiandrosterone	HPβCD	[81]
Dexamethasone	HPβCD, γCD	[82–88]
Diclofenac	HPβCD, RMβCD	[89]
Dipivefrine	SBEβCD	[90]
Disulfiram	HPβCD	[91,92]
Dorzolamide	RMβCD, γCD	[88,93]
Enalaprilat	HPβCD	[94]
Enalapril maleate	HPβCD	[94]
Ethoxyzolamide	HPβCD	[72]
Fluorometholone	HPγCD	[95]
Hydrocortisone	HPβCD	[96,97]
Ketoconazole	HPβCD	[98]
Loteprednol etabonate	HPβCD, DMβCD	[99]
Pilocarpine	αCD, βCD, HEβCD, HPβCD, SBEβCD	[100–103]
Prostaglandins	HPβCD	[104]
Rufloxacin	HPβCD	[105]
Thalidomide	HPβCD	[106]
Δ^9-Tetrahydrocannobinol	αCD	[107,108]

Table 4 Examples of cyclodextrin-containing formulations for nasal, buccal-sublingual, pulmonary, rectal and vaginal drug delivery

Drug	Cyclodextrin	Reference
Nasal drug delivery:		
Acyclovir	HPβCD	[115]
17β-Estradiol	DMβCD	[116]
Insulin	αCD, DNαCD, HPαCD, βCD, DMβCD, HPβCD	[117]
Midazolam	SBEβCD	[118,119]
Prostaglandin E_1	HPβCD	[120]
Buccal and sublingual drug delivery:		
Androstenediol	HPβCD	[121]
Atenolol	βCD, MβCD, RMβCD	[122,123]
Cannabidiol	βCD	[124]
Ciclosporin	αCD	[125]
Clomipramide	HPβCD	[126]
Danazol	HPβCD, SBEβCD	[127,128]
17β-Estradiol	HPβCD	[129–131]
Flufenamic acid	HPβCD	[132]
Δ^9-Tetrahydrocannabinol	βCD	[133]
Pulmonary drug delivery:		
Beclometasone	γCD	[134]
Budesonide	γCD	[135]
Ciclosporin	HPαCD	[136]
Itraconazole	HPβCD	[137]
Rectal drug delivery:		
Edaravone	HPβCD	[138]
Flurbiprofen	HPβCD	[139]
Vaginal drug delivery:		
Itraconazole	HPβCD	[140]
Natamycin	γCD	[141]

mixtures of CDs and drugs have less effect on oral drug bioavailability than prepared complexes, that CDs have little effect on BCS class I and II drugs, and that CDs can be used to prevent gastrointestinal absorption strongly suggest that CDs do not enhance oral drug bioavailability by decreasing the barrier function of the epithelial cell layer but act by other mechanisms.

Caco-2

The best exploited epithelial cell line for in-vitro permeation studies is Caco-2, a human colon carcinoma cell line that develops microvilli on its apical surface. In the Caco-2 permeability cell culture experimental setup, the aqueous donor phase is usually unstirred, resulting in a relatively thick UWL that forms an aqueous diffusion barrier at the apical surface of the relatively permeable cell membrane.[210,211] Studies have shown that while CDs can enhance drug permeation through cell layers, an excess of hydrophilic CD will reduce drug permeation through the cell membrane.[212–214] Lipophilic CDs, like methylated βCD, increase drug permeation through Caco-2 by depletion of cholesterol from the membrane.[215]

The parallel artificial membrane permeation assay (PAMPA)

The parallel artificial membrane permeation assay (PAMPA) is a method that determines the permeability of substances from a donor compartment, through an artificial membrane, into an acceptor compartment. The membrane consists of a microfilter disc coated with a 2% (w/v) dodecane solution of dioleylphosphadityl choline under conditions that favour formation of a multilamellar structure.[216–218] Previously, we have shown that in the PAMPA system, the thickness of the UWL and its contribution to the overall membrane barrier depends on the stirring rate.[219] In the absence of HPβCD, drug permeability increased with decreasing UWL thickness to a certain minimum values of about 40 µm. Addition of HPβCD to systems exhibiting UWL thicknesses greater than 40 µm significantly increased the drug flux through PAMPA. The effect of HPβCD appeared also to be related to the stability constant (K) of the drug/CD complex with flux increasing with increasing *K*-value.[219] This suggests that hydrophilic CDs enhance flux when the UWL resistance (i.e. the aqueous diffusion barrier) makes a significant contribution to the overall barrier resistance.

What do these studies mean?

Some general observations can be made from these studies on the effects of CDs on drug permeation through the various membrane systems (Figure 1). First, the studies have shown that CDs and their complexes do not, in general, permeate lipophilic biomembranes (i.e. their $K_{M/D} \approx 0$). The drug molecules have to be released from the complexes before they can permeate the membranes. Second, CDs are unable to enhance drug delivery from non-aqueous vehicles through biomembranes (i.e. no enhancement if no UWL is present at the membrane surface). Third, CDs do not, in general, enhance

Table 5 Examples of cyclodextrin-containing oral formulations

Drug	MW	$LogK_{o/w}$[a]	Log(S mg/ml)[b]	D : S[c] (ml)	Cyclodextrin	*Formulation*	*Species*	F_{rel}[d]	Ref.
Aciclovir	225	−1.8	0.1	10	βCD	Suspension	Rat	1.1	[149]
Albendazole	265	3.0	−3.7	10^6	HPβCD	Solution	Sheep, mouse, rabbit	≤3.2	[150–153]
Andrographolide	350	1.9	−1.2	10^2	HPβCD	Suspension	Rat	1.6	[154]
Artemisinin	284	2.3	−1.1	10^3	βCD, γCD	Capsule	Human	≤1.7	[155]
Carbamazepine	236	2.5	−0.7	10^3	DMβCD	Powder, solution, tablet	Rabbit, dog, rat	≤5.6	[156–160]
Chloramphenicol palmitate	562	1.1	0.00	10^3	HPβCD	Powder	Dog	≤3.8	[161]
Cilostazol	369	2.7	−2.5	10^4	DMβCD	Suspension	Rabbit	2.5	[162]
Cinnarizine	369	5.8	−3.0	10^5	βCD, HPβCD, SBEβCD	Tablet, solution, capsule	Dog	≤48	[163–165]
Ciclosporin	1202	4.1	−2.0	10^4	DMβCD	Suspension	Rat	4.7	[166,167]
Danazol	338	4.2	−3.3	10^4	HPβCD, SBEβCD	Capsule	Dog, rat	≤34	[127,168,169]
Dehydroepiandrosterone	288	3.2	−1.2	10	αCD	Tablet	Human	2.0	[170]
Digoxin	781	1.3	−1.2	8	γCD	Tablet	Dog	5.4	[171]
Diphenhydramine HCl	292	3.3	2.0	0.3	DMβCD, HPβCD	Solution	Rat	≤0.9	[172]
Dipyridamole	505	2.7	−2.3	10^4	βCD	Capsule	Dog, Human	≤1.6	[173,174]
Fenbufen	254	3.2	−1.5	10^2	αCD, , γCD	Suspension	Rabbit	≤5.5	[175]
Fluoxetine HCl	346	4.1	1.1	1.4	γCD	Solid dosage	Human	2.5	[176]
Flurbiprofen	244	4.2	−2.0	10^4	βCD	Suspension	Rat	1.3	[177]
Glibenclamide	494	4.8	−2.2	10^3	βCD, SBEβCD	Capsule	Dog, rat	≤6.2	[178,179]
Gliclazide	232	2.1	0.10	10^2	βCD	Suspension	Rat	6	[180]
Gliquidone	528	4.6	−0.15	10^2	HPβCD	Powder	Rat	2.0	[181]
Indometacin	358	−1.0	−0.10	10^2	E-αCD, βCD, HEβCD, HPβCD	Capsule	Human, rabbit	≤1.3	[182,183]
Ketoprofen	254	00	−1.0	10^3	βCD, HPβCD	Suspension	Rat	≤2.9	[184]
Miconazole	416	5.9	−1.4	10^3	HPβCD	Suspension	Rat	2.3	[50]
Nifedipine	346	2.2	−2.0	10^3	βCD, HPβCD	Capsule	Rabbit, dog	≤2.9	[185–188]
Phenytoin	252	2.5	−1.7	103	E-βCD, GluβCD, HPβCD, MalβCD, SBEβCD	Suspension, capsule	Rat, Dog	≤5	[189–191]
Piroxicam	331	3.1	−2.0	10^3	βCD	Tablet, capsule, suspension	Human, rat, rabbit	≤1.4	[192–196]
Raloxifene	474	5.5	−0.60	10^2	HEβCD	Capsule	Rat	3	[197]
Rofecoxib	314	2.3	−3.4	10^4	βCD	Tablet	Human	1.3	[198]
Spironolactone	417	2.8	−1.7	10^3	βCD, γCD, DMβCD, HPβCD, SBEβCD	Solution, powder	Rat, dog	≤3.6	[199–201]
Tacrolimus	804	4.0	−2.7	10^4	DMβCD, HPβCD, SBEβCD	Suspension	Rat	≤4.5	[202]
Tolbutamide	270	2.3	−1.0	10^3	βCD, HPβCD	Suspension, powder	Rabbit, dog	≤1.5	[203,204]

αCD, α-cyclodextrin; GluαCD, glucosyl-α-cyclodextrin; MalαCD, maltosyl-α-cyclodextrin; DMalαCD, dimaltosyl-α-cyclodextrin; E-αCD, α-cyclodextrin epichlorohydrin polymer; βCD, β-cyclodextrin; E-βCD, β-cyclodextrin epichlorohydrin polymer; HPβCD, 2-hydroxypropyl-β-cyclodextrin; GluβCD, glucosyl-β-cyclodextrin; MalβCD, maltosyl-β-cyclodextrin; DMβCD, dimethyl-β-cyclodextrin; TMβCD, trimethyl-β-cyclodextrin; SBEβCD, sulfobutylether-β-cyclodextrin sodium salt; HEβCD, hydroxyethyl-β-cyclodextrin; EβCD, heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin; γCD, γ-cyclodextrin. [a]$LogK_{o/w}$: the logarithm of the octanol/water partition coefficient (from SciFinder, ACS, USA (scifinder.cas.org), ChemExper Chemical Directory (http://www.chemexper.com) and [205]. [b]LogS: the logarithm of the drug solubility in water in mg/ml. [c]D : S: drug dose : solubility ratio in ml. [d]F_{rel} = relative bioavailability (i.e. the area-under-curve (AUC) of the plasma concentration versus time profile when the cyclodextrin containing formulation was given divided by the AUC for the formulation containing no cyclodextrin)

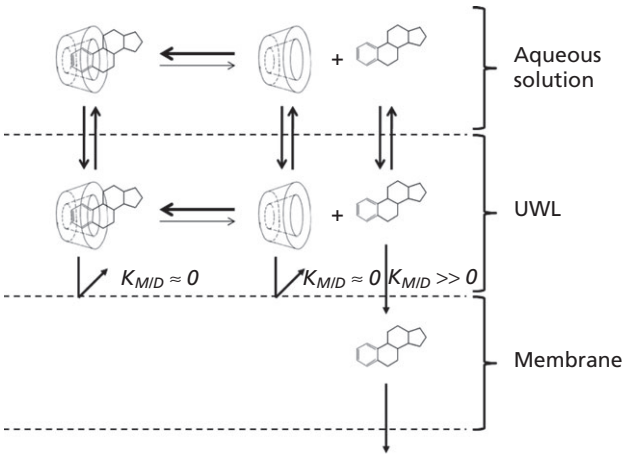


Figure 1 Scheme showing drug permeation from a vehicle consisting of a drug dissolved in an aqueous CD solution. The unstirred water layer (UWL) forms an aqueous drug diffusion barrier and the membrane surface. The membrane forms a lipophilic membrane barrier. $K_{M/D}$ is the partition coefficient of the complex, free CD or the drug between the membrane and the UWL.

membrane permeation of hydrophilic water-soluble drugs. Fourth, excess CD, more than is needed to solubilize the drug, will hamper drug permeation. Excess CD decreases the amount of free drug molecules in the UWL (Figure 1). Fifth, CDs enhance membrane permeation of lipophilic drugs when the UWL contributes to the overall barrier function of the lipophilic membrane. Sixth, CDs are able to prevent enzymatic degradation of drugs at the aqueous membrane exterior.

Membranes and permeation enhancers

Internal and external body surfaces are in most cases covered with epithelium of one type or another. In general, the epithelium consists of a collagen matrix layer and basal lamina and is covered by one or more layers of epithelial cells. Most drugs permeate epithelia cell layers via passive transport. However, numerous transport systems have been identified in almost all epithelia.[220] In skin, the outermost cells become keratinized and die. The surface of living epithelia is covered with mucus, a gel-like fluid containing mainly water (90–98%) and mucin (2–5%).[221] Mucins are large flexible glycoproteins with molecular weight ranging from 0.5 MDa to 20 MDa. Mucin forms hydrogen bonds with surrounding water molecules leading to significant increase in the thickness and the viscosity of the mucus in, for example, the gastrointestinal tract, the respiratory tract, the ocular-rhino-otolaryngeal tracts and the reproductive tract, forming an aqueous diffusion barrier to drug absorption into the body. The tear film on the eye surface (including mucus) is about 8 µm thick but the thickness of the gastrointestinal mucus layer can range from about 100 µm to 600 µm.[68,222–225] At low shear rates, the bulk viscosity of healthy human mucus is typically 1000–10 000 times greater than that of water. However, the flexible mucin chains form an aqueous matrix (hydrogel) where the micro-viscosity (i.e. the viscosity between the mucin fibres) can be as low as that of pure water.[226–229] This aqueous matrix forms an unstirred water layer (UWL) that creates an aqueous diffusion barrier that impedes drug permeation through mucosal barriers.[229] Based on determinations of diffusion constants in mucus compared with water, as a function of the hydrodynamic diameter of the diffusing particles, the mesh spacing in mucus is about 400 nm.[227]

Permeation enhancers

Most permeation enhancers enhance drug uptake through biological membranes by affecting the barrier properties of the membrane itself, either by altering the structure of the cell membrane (passive transcellular route) or by opening of the tight junctions (paracellular route).[230–232] For example, chemical enhancers, such as fatty acids, alcohols, amines and amides, permeate into the membrane where they may alter the overall solvent potential of the membrane and disrupt the ordered lipid structure within the membrane barrier thereby lowering the viscosity. These physicochemical changes facilitate drug partition from the exterior into the membrane as well as drug permeation through the membrane barrier. Physical enhancers such as ultrasound decrease the barrier function and increase the kinetic energy of drug molecules through wave energy and cavitation mechanisms while iontophoresis enhances transmembrane transport of ionized drug molecules by applying a small electrical current across the membrane barrier. All of these techniques decrease, in one way or another, the barrier property of the membrane itself. Penetration enhancers alter membrane permeation of both hydrophilic and lipophilic drugs and, in general, from both non-aqueous and aqueous donor phases. Having said this, there are other methods that do not directly affect membrane structure, including the formation supersaturated drug solutions,[233] co-administration of efflux transporter inhibitors[234] and CDs.

Thermodynamic considerations

The driving force for passive drug diffusion through the UWL or a vehicle is the gradient of the chemical potential (μ). Likewise, drug partitioning between the UWL and the membrane is controlled by the chemical potential. However, it is more common to think of diffusion and partition in terms of drug concentration. For example, according to Fick's first law the driving force for drug diffusion is the drug concentration gradient (Figure 2). Still it must be remembered that for a given vehicle or formulation, the highest drug chemical potential will result in the highest drug bioavailability.[235,236] The activity (a_2) of a drug is the product of its activity coefficient (γ_2) and its concentration in molality (m_2):

$$a_2 = \gamma_2 m_2 \tag{2}$$

and

$$\mu_2 = \mu_2^\theta + RTlna_2 = \mu_2^\theta + RTln(\gamma_2 m_2) \tag{3}$$

where μ_2 is the chemical potential of the drug, μ_2^θ is the chemical potential in a given standard state, R is the gas

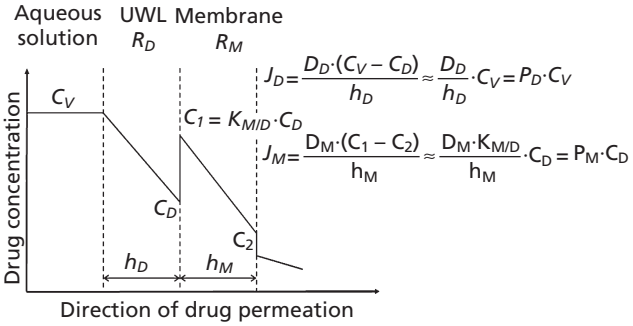


Figure 2 Scheme showing drug permeation through a simple two-layer barrier consisting of an unstirred water layer (UWL) and a lipophilic cell membrane. The aqueous solution is the vehicle containing the dissolved drug, R_D, h_D, R_M and h_M are the resistance and the thickness of the UWL (*D*) and the membrane (*M*), respectively. C_V is the drug concentration in the vehicle, C_D is the drug concentration in the UWL immediate to the membrane surface, C_1 and C_2 are the drug concentrations within the membrane at the outer and inner surface, respectively, D_D and D_M are the drug diffusion constants in the UWL and the membrane, respectively, and $K_{M/D}$ is the drug partition coefficient between the membrane and the UWL. The equations represent Fick's first law where the fluxes through the UWL (J_D) and the lipophilic membrane (J_M) are the product of drug diffusion coefficients (*D*), the drug concentration gradients and the thickness of the barriers (*h*)

constant and *T* is the temperature in Kelvin. The thermodynamic definition of the partition coefficient ($K_{o/w}$) of a drug between organic (*o*) and aqueous (*w*) phase is:

$$\frac{\mu_w^\theta - \mu_o^\theta}{RT} = ln\frac{a_o}{a_w} \approx ln\frac{\gamma_o \cdot C_o}{\gamma_w \cdot C_w} = ln\frac{\gamma_o}{\gamma_w} + lnK_{o/w} \qquad (4)$$

Equation 4 states that equilibrium between the two phases is attained when the chemical potential of the drug in one phase (e.g. the aqueous membrane exterior) is equal to the chemical potential in the other phase (e.g. the membrane itself). Thermodynamic activity is equal to unity in saturated solutions and, thus, many ointments and creams consist of finely divided drug suspensions. In this situation the drug has the highest potential to leave the vehicle and permeate into and through the membrane barrier. The activity is greater than unity in supersaturated solutions but these are physically metastable states. Addition of solubilizers, such as CDs, to an aqueous drug solution will lower the drug activity (i.e. lowers γ_w in Equation 4) and, thus, the potential of the drug to exit the formulation.[237] Addition of solubilizers to aqueous drug suspension, increasing the amount of dissolved drug while keeping the solution saturated with drug, will not lower the drug activity. Under such condition the thermodynamic activity will remain equal to unity and, thus, the dissolved drug molecules are at their highest 'exiting' potential while the total amount of dissolved drug is increased.

Theoretical background

Most biological membranes are multilayer barriers and most contain various transport systems. In addition, the majority of drugs permeate biological membranes via passive diffusion and all drugs by definition permeate the UWL (the aqueous diffusion barrier at the membrane surface) via passive diffusion. Even drugs that are carried through membranes by some active membrane transporters must passively permeate the UWL to reach the transporters. The UWL can be as thick as 100 μm (or even more), in the human small intestine, or as thin as a fraction of μm on, for example, the skin surface.[222] Under in-vitro conditions, the thickness of the UWL can be well above 1000 μm in an unstirred aqueous donor phase and *in vivo* its thickness is frequently 10–100 μm. However, the thickness of the UWL depends also on the physicochemical properties of the permeating drug molecule, including its ability to form ionic and hydrogen bonds with mucin, and thus a fixed UWL thickness for all drugs does not exist.[238]

In the following section it is assumed that a biological membrane consists of only two layers (i.e. an UWL and a lipophilic cell membrane). Furthermore it is assumed that drug molecules permeate both these layers via passive diffusion. The mathematical model is based on work by Higuchi,[235] Zwolinski, Eyring and Reese[239] and Flynn and Yalkowsky.[240,241] Assuming independent and additive resistances of the two individual layers, the total resistance (R_T) of a simple membrane (Figure 2) can be defined as:

$$R_T = R_D + R_M \qquad (5)$$

where R_D and R_M are the resistances in the UWL at the exterior and within the membrane, respectively. Since the permeability constants (*P*) are the reciprocals of the resistances, the following equation is obtained, assuming sink conditions (i.e. $C_V - C_D \approx C_V$ and $C_1 - C_2 \approx C_1$ in Figure 2):

$$J = P_T \cdot C_V = (R_D + R_M)^{-1} \cdot C_V = \left(\frac{1}{P_D} + \frac{1}{P_M}\right)^{-1} \cdot C_V \qquad (6)$$

where *J* is the flux of the drug through the membrane, P_T is the overall permeability coefficient, C_V is the concentration of the compound in the vehicle (i.e. donor phase), and P_D and P_M are the permeability coefficients in the UWL at the donor side and within the membrane, respectively. Rearranging Equation 6 gives:

$$J = \left(\frac{P_D \cdot P_M}{P_D + P_M}\right) \cdot C_V \qquad (7)$$

If permeation is much slower through the membrane itself than the UWL (i.e. $P_D > P_M$), then Equation 7 becomes:

$$J = \left(\frac{P_D \cdot P_M}{P_D + P_M}\right) \cdot C_V \approx \left(\frac{P_D \cdot P_M}{P_D}\right) \cdot C_V = P_M \cdot C_V \qquad (8)$$

In that case, the UWL only has negligible effect on the drug permeation through the membrane and can be ignored (i.e. $R_M > R_D$). This can, for example, be the case when relatively large and/or hydrophilic molecules permeate mucosa

(e.g. some BCS Class III drugs). If, on the other hand, permeation through the membrane is much faster than permeation through the UWL (i.e. $P_M > P_D$) Equation 7 becomes:

$$J = \left(\frac{P_D \cdot P_M}{P_D + P_M}\right) \cdot C_V \approx \left(\frac{P_D \cdot P_M}{P_M}\right) \cdot C_V = P_D \cdot C_V \qquad (9)$$

In this case the UWL is the main barrier (i.e. $R_D > R_M$) and drug permeation through the membrane becomes aqueous diffusion layer-controlled. This can for example be the case when relatively small and lipophilic molecules permeate mucosa (e.g. some BCS Class II drugs). The relationship between the permeation coefficient (P) and the diffusion coefficient (D) is given by Equation 10:

$$P = \frac{D \cdot K}{h} \qquad (10)$$

where h is the thickness (h_D or h_M in Figure 2) and K is the partition coefficient between the aqueous phase and the membrane. For P_D value of K is unity. Finally D can be estimated from the Stokes–Einstein equation:

$$D \approx \frac{R \cdot T}{6\pi \cdot \eta \cdot r \cdot N} \qquad (11)$$

where R is the molar gas constant, T is the absolute temperature, η is the apparent viscosity within the UWL or the lipophilic membrane, r is the radius of the permeating drug molecule and N is Avogadro's number. Thus, the diffusion constant within the UWL (D_D) will decrease with increasing viscosity of the layer as well as with increasing molecular weight of the permeating drug. Mucus on the surface of the epithelial cell layer forms a kind of hydrogel on a lipophilic surface where stagnant aqueous domains are located within a polymer matrix. Mucus will increase the thickness (h_D) of the UWL and increased viscosity (η) will decrease the value of D_D that again will decrease the value of P_D and increase the value of R_D.

Figure 3 shows the effect of relative drug-CD concentration on the ability of a poorly soluble lipophilic drug to permeate a biological membrane where the UWL forms an aqueous diffusion barrier at the membrane surface. In other words, where the drug flux through the membrane follows Equation 9. In this example, the total amount of drug in the aqueous donor phase (the vehicle) is kept constant but the concentration of CD is between zero and twice the amount needed to solubilize the drug in the donor phase. The vertical broken line through the middle of the figure denotes the CD concentration that is needed to solubilize the entire amount of drug that is present in the donor phase. At lower CD concentrations, the drug is in a suspension but in solution at higher concentrations. At CD concentrations below the broken line, the donor phase consists of drug saturated CD solution but unsaturated solution at higher CD concentrations. The total concentration (S_{tot}) of dissolved drug (i.e. both free ([drug]) and dissolved in a drug/CD complex ([drug/CD])) increases

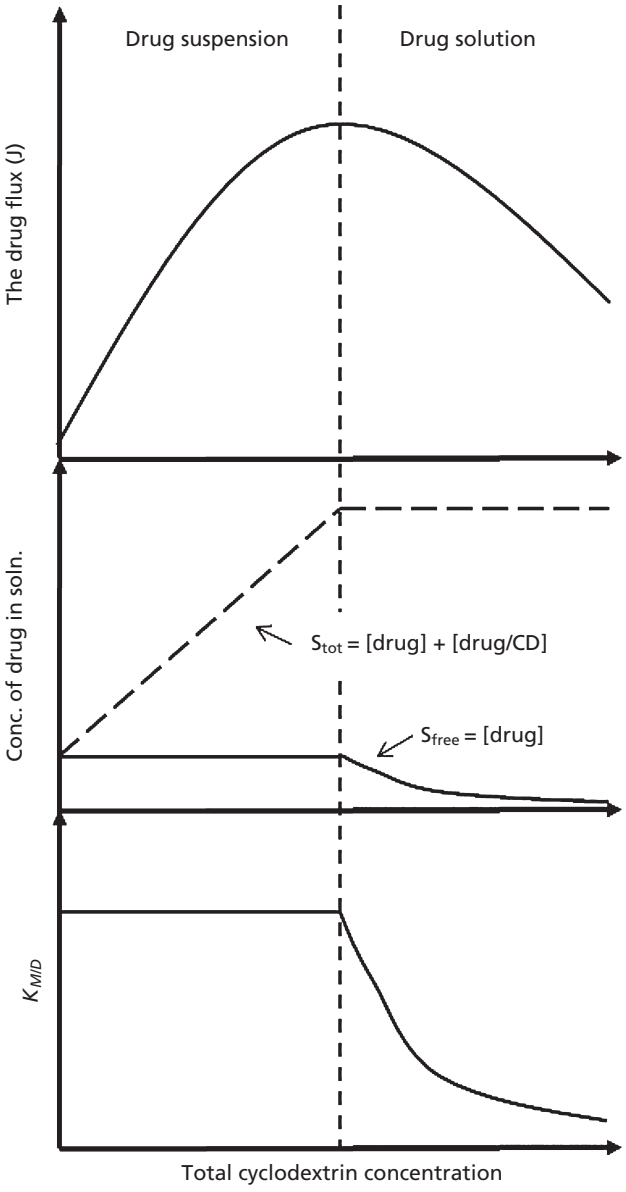


Figure 3 The effect of CD solubilization on drug permeation from an aqueous donor phase through a biological membrane where the UWL is the rate-limiting barrier. The drug concentration is kept constant and the CD is increased in such a way that at low CD concentration the drug is only partly solubilized in the donor phase but excess CD, more than is needed to solubilize the drug, is present in the donor phase at high CD concentration. Top: the drug flux (J) through the membrane; middle: the total concentration of dissolved drug (S_{tot}) in the donor phase and the concentration of dissolved free drug (i.e. that is not in a CD complex (S_{free})) in the donor phase; bottom: the observed drug partition coefficient between the lipophilic membrane and its aqueous exterior (i.e. the aqueous diffusion layer ($K_{M/D}$)).

as the CD concentration increases until all drug in the donor phase has been dissolved (see Figure 3, middle). After that point, the concentration remains constant. However, the concentration of free drug (S_{free}) will be affected. It is constant and equal to the intrinsic solubility (S_0) in drug-saturated CD solutions but decreases as the solution becomes unsaturated at

CD concentrations above the broken vertical line. Below the line, the activity of the drug (a_2 in Equations 2 and 3; a_W in Equation 4) is equal to unity but decreases as the CD concentration increases above the broken vertical line. As the aqueous drug donor solution becomes more unsaturated, the value of γ_D (i.e. γ_w in Equation 4) becomes smaller. This change in γ_D will affect the observed drug partition coefficient ($K_{M/D}$) between the lipophilic membrane and its aqueous exterior (see Figure 2):

$$\frac{\mu_D^\theta - \mu_M^\theta}{RT} - ln\frac{Y_M}{Y_D} \approx lnK_{M/D} \qquad (12)$$

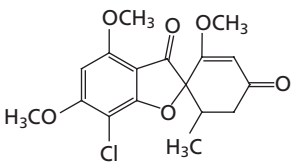
$K_{M/D}$ remains constant as long as activity (i.e. $a_D = \gamma_D \cdot C_D$) remains constant but decreases as the value of γ_D decreases (Figure 3, bottom). Thus, when CD is added as a solubilizer to aqueous formulations to enhance drug delivery through a membrane where the UWL forms an aqueous diffusion barrier and the flux follows Equation 9, the following will be observed:

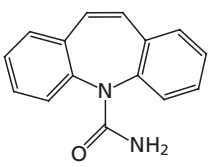
- When excess solid drug is present (i.e. aqueous drug suspension), adding CD to the donor phase will increase the concentration gradient over the UWL (i.e. $C_V - C_D$ in Figure 2) and consequently the flux (i.e. J_D in Figure 2) will increase. This will increase the overall availability of drug (i.e. both free drug and drug/CD complex) at the membrane surface and, since the drug release from the drug/CD complex is much faster than permeation of free drug through the UWL, the increase in drug/CD complex availability will increase drug delivery through the membrane.
- If all drug is in solution, addition of CD to the donor phase will decrease the availability of free drug at the aqueous membrane exterior. Also, increasing the CD concentration will decrease the value of γ_D (i.e. the aqueous solution will be less saturated). This will decrease the $K_{M/D}$-value and, consequently, decreases the drug flux (J_M) through the membrane (see Figure 2). When excess CD is present the permeation switches from a diffusion-controlled to a membrane-controlled (i.e. $P_D > P_M$) process.
- Maximum drug flux through the membrane is obtained when just enough CD is added to solubilize the entire drug in the aqueous donor phase (at the broken vertical line in Figure 3). At that CD concentration, the concentration of dissolved drug is at its maximum and at the same time the drug has its highest potential to leave the vehicle and permeate into and through the membrane barrier. Consequently, at this CD concentration the drug bioavailability is at its maximum.

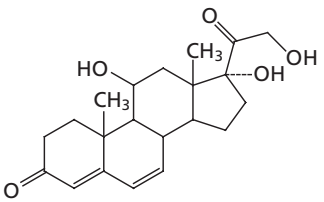
Formulation with CDs

The effect of CDs on drug permeation depends also on the interaction between the drug and the CD. The effect of HPβCD on the flux (J) of three different drugs was determined through PAMPA membrane (Table 6).[219] In the PAMPA, it was found that a UWL thickness (h_D) of 40 μm or less did not affect the drug permeation through the membrane while an h_D of 100 μm or more had significant effect. In other words, when no CD was present, the permeation was membrane-controlled at $h_D \leq 40$ μm but diffusion-controlled at $h_D \geq 100$ μm. The three drugs have comparable physicochemical properties but different affinities towards HPβCD with $K_{1:1}$ ranging from 23 M^{-1} to 1340 M^{-1}. In the absence of HPβCD, the value of J was two- to four-fold larger at h_D of 40 μm than at $h_D > 100$ μm. Addition of HPβCD decreases the effect of the UWL but the effect depended on the ability of drug from complex with HPβCD. Thus, HPβCD had no effect

Table 6 The physicochemical properties of griseofulvin, carbamazepine and hydrocortisone and the effect of the stability constant of the 1 : 1 drug/HPβCD complex ($K_{1:1}$) and thickness of the UWL (h_D) on the flux in the PAMPA system







Properties	Griseofulvin		Carbamazepine		Hydrocortisone	
Molecular weight of anhydrous compound (Da)[a]	352.8		236.3		362.5	
Melting point (°C)[a]	217–224		189–193		214	
Calculated $LogK_{(octanol/water)}$[a]	2.2		2.5		1.6	
Solubility in water at 25°C (mg/ml)[b]	0.03		0.2		0.3	
Apparent $K_{1:1}$ at room temperature (M^{-1})[b]	23		650		1340	
Flux (J) × 10^6 at room temperature (mg cm^{-2} s^{-1})[b]	$h_D > 100$ μm	$h_D = 40$ μm	$h_D > 100$ μm	$h_D = 40$ μm	$h_D > 100$ μm	$h_D = 40$ μm
0% (w/v) HPβCD	0.095 ± 0.014	0.254 ± 0.037	3.56 ± 0.27	12.5 ± 0.9	4.82 ± 1.09	11.7 ± 0.7
1% (w/v) HPβCD	0.433 ± 0.034	1.55 ± 0.31	8.83 ± 0.90	12.4 ± 0.6	15.7 ± 6.1	12.8 ± 0.2
5% (w/v) HPβCD	0.710 ± 0.261	2.38 ± 0.38	14.1 ± 6.3	15.2 ± 4.2	24.8 ± 9.8	15.0 ± 2.3
10% (w/v) HPβCD	–	–	17.3 ± 4.6	17.3 ± 4.6	19.0 ± 4.4	14.6 ± 4.4

[a]From [205]. [b]From [219]. The aqueous pH 7.4 donor phase solutions containing from 0 to 10% (w/v) HPβCD were saturated with the drug to be tested.

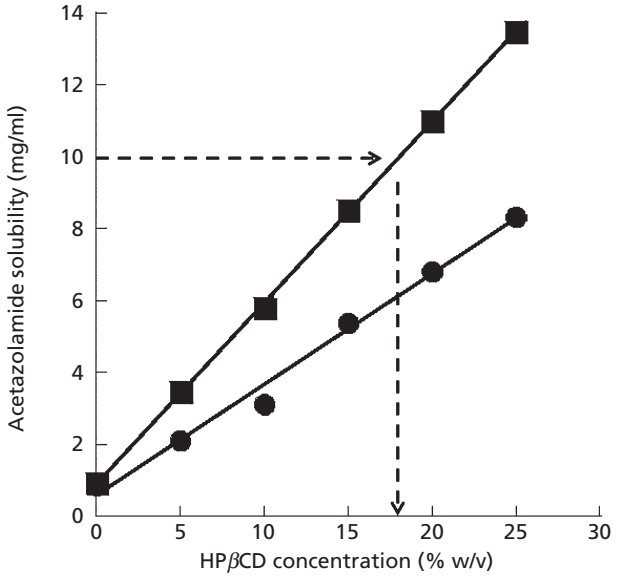


Figure 4 Phase-solubility of acetazolamide in pure aqueous HPβCD solution (●), $K_{1:1} = 20\ M^{-1}$, and in aqueous eye drop formulation (■) containing, in addition to HPβCD, 0.02% (w/v) benzalkonium chloride, 0.05% (w/v) EDTA, 0.5% (w/v) hydroxypropyl methylcellulose and enough sodium chloride to make the solution isotonic, $K_{1:1} = 54\ M^{-1}$. Partly based on [72,242]

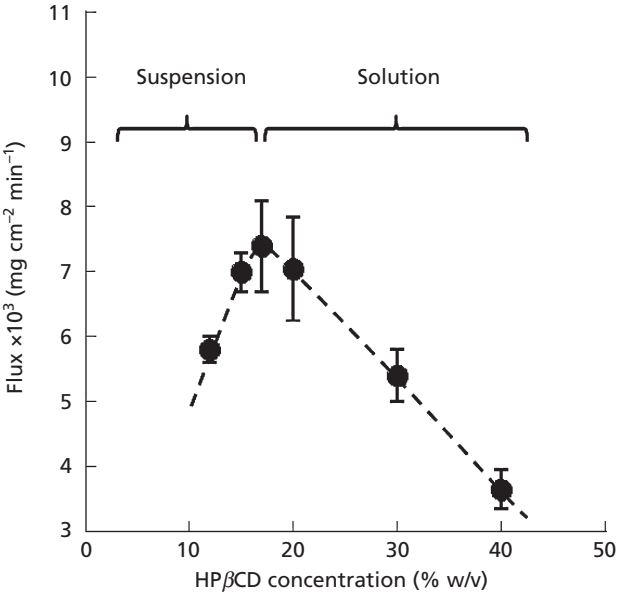


Figure 5 The relationship between HPβCD concentration in the aqueous eye drop formulation and the flux (J) of acetazolamide from an aqueous eye drop formulation. The concentration of acetazolamide was kept constant at 1.0% (w/v) but the HPβCD concentration ranged from 12% to 40% (w/v). Modified from reference[242]

on the J of griseofulvin ($K_{1:1} = 23\ M^{-1}$) through the PAMPA membrane, had a significant effect on carbamazepine ($K_{1:1} = 650\ M^{-1}$) and a very significant effect on hydrocortisone ($K_{1:1} = 1340\ M^{-1}$). The effect increased with increasing $K_{1:1}$-value.

Since the availability of a drug depends on the ability of the drug molecules to interact with the CD molecules and the drug : CD concentration ratio and since the drug–CD interaction is affected by other excipients present in the drug formulation, it is of uttermost importance to optimize the final drug formulation with regard to the amount of CD. Too much or too little CD will result in less than optimal drug availability. Acetazolamide was formulated as an aqueous 1.0% (w/v) eye drop solution.[242] The HPβCD solubilization of acetazolamide was enhanced by the excipients commonly used in eye drops (i.e. the preservatives and hydroxypropyl methylcellulose), and consequently about 40% less HPβCD was needed to solubilize the drug in the aqueous eye drop formulation than in pure water (Figure 4). The release of acetazolamide was also influenced by the HPβCD concentration (Figure 5). Maximum release was obtained when just enough CD was used to solubilize the drug (18% (w/v) HPβCD). This low-viscosity eye drop solution containing 1.0% (w/v) acetazolamide and 18% (w/v) HPβCD had a notable intraocular pressure lowering effect in rabbits.[72,242]

Conclusions

In general, CDs enhance drug delivery through biological membranes by increasing drug permeation through the UWL (i.e. by increasing the availability of dissolved drug molecules juxtaposed to the membrane surface). CDs only enhance drug permeation when UWL is present at the membrane exterior. This UWL can consist of mucus or an aqueous vehicle such as o/w creams or hydrogels, or simply as unstirred aqueous donor phases in in-vitro experiments. CDs do not enhance drug permeation from vehicles that do not form an UWL, such as ointments and w/o creams. The effect also depends on the physicochemical properties of the drug. Better enhancement is obtained for lipophilic drugs that are poorly soluble in water and that form water-soluble complexes with CDs with stability constants ($K_{1:1}$) that are between about 50 M^{-1} and 5000 M^{-1}. Finally, it is of uttermost importance to optimize the drug vehicle with regard to the amount of CD. Too much or too little CD will result in less than optimal drug bioavailability.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This review received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

1. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins: basic science and product development. *J Pharm Pharmacol* 2010; 62: 1607–1621.
2. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. *J Pharm Sci* 1996; 85: 1017–1025.

3. Sabadini E *et al*. Solubility of cyclomaltooligosaccharides (cyclodextrins) in H_2O and D_2O: a comparative study. *Carbohydr Res* 2006; 341: 270–274.
4. Antlsperger G, Schmid G. Toxicological comparison of cyclodextrins. In: Szejtli J, Szente L, eds. *Proceedings of the Eighth International Symposium on Cyclodextrins. Budapest, Hungary, March 32 – April 2, 1996*. Dordrecht: Kluwer Acad. Pub, 1996: 149–155.
5. Antlsperger G. New aspects in cyclodextrin toxicology. In: Hedges AR, ed. *Minutes of the Sixth International Symposium on Cyclodextrins*. Paris: Editions de Santé, 1992: 277–283.
6. Pitha J *et al*. Hydroxypropyl-β-cyclodextrin: preparation and characterization; effects on solubility of drugs. *Int J Pharm* 1986; 29: 73–82.
7. Buedenbender S, Schulz GE. Structural base for enzymatic cyclodextrin hydrolysis. *J Mol Biol* 2009; 385: 606–617.
8. Szejtli J. The metabolism, toxicity and biological effects of cyclodextrins. In: Duchêne D, ed. *Cyclodextrins and Their Uses*. Paris: Editions de Santé, 1987: 173–212.
9. Munro IC *et al*. Safety assessment of γ-cyclodextrin. *Regul Toxicol Pharmacol* 2004; 39(Suppl. 1): S3–S13.
10. Irie T, Uekama K. Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. *J Pharm Sci* 1997; 86: 147–162.
11. Stella VJ, He Q. Cyclodextrins. *Tox Pathol* 2008; 36: 30–42.
12. De Bie ATHJ *et al*. Disposition of 14C-γ-cyclodextrin in germ-free and conventional rats. *Regul Toxicol Pharmacol* 1998; 27: 150–158.
13. Van Ommen B *et al*. Disposition of 14C-α-cyclodextrin in germ-free and conventional rats. *Regul Toxicol Pharmacol* 2004; 39: S57–S66.
14. Zhou H *et al*. A pharmacokinetic study of intravenous intraconazole followed by oral administration of intraconazole capsules in patients with advanced human immunodeficiency virus infection. *J Clin Pharmacol* 1998; 38: 593–602.
15. Stella VJ *et al*. Mechanism of drug release from cyclodextrin complexes. *Adv Drug Deliv Rev* 1999; 36: 3–16.
16. Adam JM *et al*. Cyclodextrin-derived host molecules as reversal agents for the neuromuscular blocker rocuronium bromide: synthesis and structure-activity relationships. *J Med Chem* 2002; 45: 1806–1816.
17. Welliver M. New drug sugammadex: a selective relaxant binding agent. *AANA J* 2006; 74: 357–363.
18. Perry CS *et al*. The binding interaction of synthetic ozonide antimalarials with natural and modified β-cyclodextrins. *J Pharm Sci* 2006; 95: 146–158.
19. Lipinski CA. Drug-like properties and the cause of poor solubility and poor permeability. *J Pharmacol Toxicol Methods* 2000; 44: 235–249.
20. Lipinski CA *et al*. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 2001; 46: 3–26.
21. Loftsson T *et al*. Improved acitretin delivery through hairless mouse skin by cyclodextrin complexation. *Int J Pharm* 1995; 115: 255–258.
22. Chen C-Y *et al*. Effect of hydroxypropyl-β-cyclodextrin on the solubility, photostability and in-virto permeability of alkannin/shikonin enantiomers. *Int J Pharm* 1996; 141: 171–178.
23. Yang J *et al*. Influence of hydroxypropyl-β-cyclodextrin on transdermal penetration and photostability of avobenzone. *Eur J Pharm Biopharm* 2008; 69: 605–612.
24. Uekama K *et al*. Improvement in the percutaneous absorption of beclomethasone dipropionate by γ-cyclodextrin complexation. *J Pharm Pharmacol* 1985; 37: 532–535.
25. Arima H *et al*. Possible enhancing mechanism of the cutaneous permeation of 4-biphenylylacetic acid by β-cyclodextrin derivatives in hydrophilic ointment. *Chem Pharm Bull* 1996; 44: 582–586.
26. Arima H *et al*. Enhancement of antiinflammatory effect of ethyl 4-biphenylyl acetate in ointment by β-cyclodextrin derivatives: increased absorption and localized activation of the prodrug in rats. *Pharm Res* 1990; 7: 1152–1156.
27. Babu RJ, Pandit JK. Effect of cyclodextrins on the complexation and transdermal delivery of bupranolol through rat skin. *Int J Pharm* 2004; 271: 155–165.
28. Zi P *et al*. Effect of HPβCD on solubility and transdermal delivery of capsaicin through rat skin. *Int J Pharm* 2008; 358: 151–158.
29. Ventura CA *et al*. Influence of modified cyclodextrins on solubility and percutaneous absorption of celecoxib through human skin. *Int J Pharm* 2006; 314: 37–45.
30. Hegge AB *et al*. *In vitro* release of curcumin from vehicles containing alginate and cyclodextrin. Studies of curcumin and curcuminoides. XXXIII. *Pharmazie* 2008; 63: 585–592.
31. Lopez RFL *et al*. Influence of cyclodextrin complexation on the in vitro permeation and skin metabolism of dexamethasone. *Int J Pharm* 2000; 200: 127–132.
32. Loftsson T *et al*. The effects of cyclodextrins on transdermal delivery of drugs. *Eur J Pharm Biopharm* 1991; 37: 30–33.
33. Kear CL *et al*. Investigation into the mechanism by which cyclodextrins influence transdermal drug delivery. *Drug Dev Ind Pharm* 2008; 34: 692–697.
34. Preiss A *et al*. Penetration of hydrocortisone into excised human skin under the influence of cyclodextrins. *Pharmazie* 1995; 50: 121–126.
35. Chang SL, Banga AK. Transdermal iontophoretic delivery of hydrocortisone from cyclodextrin solutions. *J Pharm Pharmacol* 1998; 50: 635–640.
36. Loftsson T *et al*. The influence of 2-hydroxypropyl-β-cyclodextrin on diffusion rates and transdermal delivery of hydrocortisone. *Drug Dev Ind Pharm* 1994; 20: 1699–1708.
37. Loftsson T, Sigurðardottir AM. The effect of polyvinylpyrrolidone and hydroxypropyl methylcellulose on HPβCD complexation of hydrocortisone and its permeability through hairless mouse skin. *Eur J Pharm Sci* 1994; 2: 297–301.
38. Sigurdardottir AM, Loftsson T. The effect of polyvinylpyrrolidone on cyclodextrin complexation of hydrocortisone and its diffusion through hairless mouse skin. *Int J Pharm* 1995; 126: 73–78.
39. Masson M *et al*. Cyclodextrins as permeation enhancers: some theoretical evaluations and *in vitro* testing. *J Controlled Release* 1999; 59: 107–118.
40. Iervolino M *et al*. Membrane penetration enhancement of ibuprofen using supersaturation. *Int J Pharm* 2000; 198: 229–238.
41. Okamoto H *et al*. Effects of β-cyclodextrin and di-O-methyl-β-cyclodextrin on the percutaneous absorption of butylparaben, indomethacin and sulfanilic acid. *Int J Pharm* 1986; 30: 35–45.
42. Kawahara K *et al*. Effect of diethyl β-cyclodextrin on the release and absorption behaviour of indomethacin from ointment bases. *STP Pharma Sci* 1992; 2: 506–513.
43. Batzdorf T, Mullergoymann CC. Release of ketoprofen from aqueous systems in the presence of hydrophilic β–cyclodextrin derivatives. *Pharmazutische Ind* 1993; 55: 857–860.
44. Vollmer V *et al*. In vivo skin pharmacokinetics of liarozole: percutaneous absorption studies with different formulations of cyclodextrin derivatives in rats. *Int J Pharm* 1993; 99: 51–58.
45. Dollo G *et al*. Complexation between local anaesthetics and β-cyclodextrin derivatives – ralationship between stability con-

stants and in vitro membrane permeability of bupivacaine and lidocaine from their complexes. *STP Pharma Sci* 1998; 8: 189–195.
46. Loftsson T, Bodor N. The pharmacokinetics and transdermal delivery of loteprednol etabonate and related soft steroids. *Adv Drug Deliv Rev* 1994; 14: 293–299.
47. Lee BJ *et al*. Percutaneous absorption and model membrane variations of melantonin in aqueous-based propylene glycol and 2-hydroxypropyl-β-cyclodextrin vehicles. *Arch Pharm Res* 1998; 21: 503–507.
48. Bounoure F *et al*. Effect of partially methylated β cyclodextrin on percutaneous absorption of metopimazine. *J Incl Phenom Macroc Chem* 2007; 57: 191–195.
49. Tanaka M *et al*. Effect of 2-hydroxypropyl-β-cyclodextrin on percutaneous absorption of methyl paraben. *J Pharm Pharmacol* 1995; 47: 897–900.
50. Tenjarla S *et al*. Preparation, characterization, and evaluation of miconazole-cyclodextrin complexes for improved oral and topical delivery. *J Pharm Sci* 1998; 87: 425–429.
51. Celebi N *et al*. The effect of β-cyclodextrin and penetration additives on the release of naproxen from ointment bases. *Pharmazie* 1993; 48: 914–917.
52. Legendre JY *et al*. Effects of β-cyclodextrins on skin: implications for the transdermal delivery of piribedil and a novel cognition enhancing-drug, S-9977. *Eur J Pharm Sci* 1995; 3: 311–322.
53. Doliwa A *et al*. In vitro iontophoretic delivery of piroxicam from hydroxypropyl-β-cyclodextrin – pyroxicam complexes. In: *Proceedings of the 27th International Symposium on Controlled Release Bioactive Materials* Paris: CRS, 2000: 7408.
54. Doliwa A *et al*. Influence of piroxicam : hydroxypropyl-beta-cyclodextrin complexation on the in vitro permeation and skin retention of piroxicam. *Skin Pharmacol Appl Skin Physiol* 2001; 14: 97–107.
55. Uekama K *et al*. Improvement of percutaneous absorption of prednisolone by β- and γ-cyclodextrin complexations. *Chem Pharm Bull* 1987; 35: 2910–2913.
56. Adachi H *et al*. Inhibitory effect of prostaglandin E1 on laurate-induced peripheral vascular occlusive sequelae in rabbits; optimized topical formulation with β-cyclodextrin derivative and penetration enhancer HPE-101. *J Pharm Pharmacol* 1992; 44: 1033–1035.
57. Uekama K *et al*. Improved transdermal delivery of prostaglandin E_1 through hairless mouse skin: combined use of carboxymethyl-ethyl-β-cyclodextrin and penetration enhancers. *J Pharm Pharmacol* 1992; 44: 119–121.
58. Adachi H *et al*. Combination effects of *O*-carboxymethyl-*O*-ethyl-β-cyclodextrin and penetration enhancer HPE-101 on transdermal delivery of prostaglandin-E(1) in hairless mice. *Eur J Pharm Sci* 1993; 1: 117–123.
59. Yuzuriha S *et al*. Topical application of prostaglandine E1 ointment to cutaneous wounds in ischemic rabbit ears. *Eur J Plast Surg* 1999; 22: 225–229.
60. Szeman J *et al*. Enhanced percutaneous absorption of homogenized tolnaftate/β-cyclodextrin polymer ground mixture. *Drug Des Deliv* 1987; 1: 325–332.
61. Amdidouche D *et al*. Evaluation by laser-doppler velocimetry of the attenuation of tretioin induced skin irritation by β-cyclodextrin complexation. *Int J Pharm* 1994; 111: 111–116.
62. Montassier P *et al*. In vitro release study of tretinoin from tretinoin/cyclodextrin derivative complexes. *J Inclusion Phenom Mol Recog Chem* 1998; 31: 213–218.
63. Gerlóczy A *et al*. Percutaneous absorption of heptakis-(2,6-di-O-14C-methyl)-β-cyclodextrin in rats. In: Huber O, Szejtli J, eds. *Proceedings of the Fourth International Symposium on Cyclodextrins*. Dordrecht: Kluwer Academic Publishers, 1988: 415–420.
64. Loftsson T, Masson M. Cyclodextrins in topical drug formulations: theory and practice. *Int J Pharm* 2001; 225: 15–30.
65. Vitória M *et al*. Characterization of the influence of some cyclodextrins on the stratum corneum from the hairless mouse. *J Pharm Pharmacol* 1997; 49: 397–402.
66. Arima H *et al*. Enhancing effect of hydroxypropyl-β-cyclodextrin on cutaneous penetration activation of ethyl 4-biphenylyl acetate in hairless mouse skin. *Eur J Pharm Sci* 1998; 6: 53–59.
67. Preiss A *et al*. In-vitro hydrocortisone release from ointments in presence of cyclodextrins. *Pharmazie* 1994; 49: 902–906.
68. Loftsson T *et al*. Effects of cyclodextrins on drug delivery through biological membranes. *J Pharm Sci* 2007; 96: 2532–2546.
69. Felton LA *et al*. Influence of hydroxypropyl-β-cyclodextrin on transdermal permeation and skin accumulation of oxybenzone. *Drug Dev Ind Pharm* 2002; 28: 1117–1124.
70. Sarveiya V *et al*. Inclusion complexation of the sunscreen 2-hydroxy-4-methoxy benzophenone (oxybenzone) with hydroxypropyl-β-cyclodextrin: effect on membrane diffusion. *J Incl Phenom Macroc Chem* 2004; 49: 275–281.
71. Felton LA *et al*. Influence of cyclodextrin complexation on the in vivo photoprotective effects of oxybenzone. *Drug Dev Ind Pharm* 2004; 30: 95–102.
72. Loftsson T *et al*. Topically effective ocular hypertensive acetazolamide and ethoxyzolamide formulations in rabbits. *J Pharm Pharmacol* 1994; 46: 503–504.
73. Loftsson T *et al*. Topically effective acetazolamide eye-drop solution in man. *Pharm Sci* 1996; 2: 277–279.
74. Granero GE, Longhi MR. Promishing complexes of acetazolamide for topical ocular administration. *Expert Opin Drug Deliv* 2010; 7: 943–953.
75. Jarho P *et al*. Increase in aqueous solubility, stability and in vitro corneal permeability of anandamide by hydroxypropyl-β-cyclodextrin. *Int J Pharm* 1996; 137: 209–217.
76. Pate DW *et al*. Effects of topical anandamides on intraocular pressure in normotensive rabbits. *Life Sci* 1996; 58: 1849–1860.
77. Pate DW *et al*. Effect of the CB 1 receptor antagonist, SR 141716A, on cannabinoid-induced ocular hypertension in normotensive rabbits. *Life Sci* 1998; 63: 2181–2188.
78. Cheeks L *et al*. Influence of vehicle and anterior chamber protein concentration on cyclosporin penetration through the isolated rabbit cornea. *Curr Eye Res* 1992; 11: 641–649.
79. Kanai A *et al*. The effect on the cornea of alpha cyclodextrin vehicle for cyclosporin eye drops. *Transplant Proc, book1* 1989; 21: 3150–3152.
80. Sasamoto Y *et al*. Topical application of ciclosporin ophthalmic solution containing alpha-cyclodextrin in experimental uveitis. *Ophthalmologica* 1991; 203: 118–125.
81. Kearse EC *et al*. Influence of dehydroepiandrosterone on rabbit intraocular pressure. *Ophthalmic Res* 2001; 33: 42–47.
82. Usayapant A *et al*. Effect of 2-hydroxypropyl-β-cyclodextrin on the ocular absorption of dexamethasone and dexamethasone acetate. *Pharm Res* 1991; 12: 1495–1499.
83. Loftsson T *et al*. The effect of hydroxypropyl methylcellulose on release of dexamethasone from aqueous 2-hydroxypropyl-β-cyclodextrin formulations. *Int J Pharm* 1994; 104: 181–184.
84. Kristinsson JK *et al*. Dexamethasone-cyclodextrin-polymer co-complexes in aqueous eye drops. *Invest Ophthalmol Vis Sci* 1996; 37: 1199–1203.

85. Gavrilin MV *et al*. Dexamethasone eye drops based on the products of its interaction with 2-hydroxypropyl-β-cyclodextrin: synthesis and study. *Pharm Chem J* 1999; 33: 160–163.
86. Loftsson T *et al*. Dexamethasone delivery to posterior segment of the eye. *J Incl Phenom Macroc Chem* 2007; 57: 585–589.
87. Loftsson T *et al*. Cyclodextrin microparticles for drug delivery to the posterior segment of the eye: aqueous dexamethasone eye drops. *J Pharm Pharmacol* 2007; 59: 629–635.
88. Sigurdsson HH *et al*. Topical and systemic absorption in delivery of dexamethasone to the anterior and posterior segments of the eye. *Acta Ophthalmol Scand* 2007; 85: 598–602.
89. Reer O *et al*. In vitro corneal permeability of diclofenac sodium in formulations containing cyclodextrins compared to commercial product voltaren ophtha. *J Pharm Sci* 1994; 83: 1345–1349.
90. Jarho P *et al*. The use of cyclodextrins in ophthalmic formulations of dipivefrin. *Int J Pharm* 1997; 153: 225–233.
91. Nagai N *et al*. Delay in ICR/f rat lens opacification by the instillation of eye drops containing disulfiram and hydroxypropyl-β-cyclodextrin inclusion complex. *Biol Pharm Bull* 2007; 30: 1529–1534.
92. Ito Y *et al*. Reduction of intraocular pressure by the installation of eye drops containing disulfiram induced with 2-hydroxypropyl-β-cyclodextrin in rabbits. *Biol Pharm Bull* 2010; 33: 1574–1578.
93. Jansook P *et al*. Cyclodextrin solubilization of carbonic anhydrase inhibitor drugs: formulation of dorzolamide eye drop microparticle suspension. *Eur J Pharm Biopharm* 2010; 76: 208–214.
94. Loftsson T *et al*. Enalaprilat and enalapril maleate eye drops lower intraocular pressure in rabbits. *Acta Ophthalmol Scand* 2010; 88: 337–341.
95. Morita Y *et al*. Effect of hydroxypropyl-γ-cyclodextrin on ocular penetration of fluorometholone in vitro. In: *23rd International Symposium on Controlled Release Bioactive Materials.* Kyoto, Japan: Controlled Release Society, 1996: 451–452.
96. Bary AR *et al*. Considerations in the use of hydroxypropyl-β-cyclodextrin in the formulation of aqueous ophthalmic solutions of hydrocortisone. *Eur J Pharm Biopharm* 2000; 50: 237–244.
97. Davies NM *et al*. Evaluation of a hydrocortisone/hydroxypropyl-β-cyclodextrin solution for ocular drug delivery. *Int J Pharm* 1997; 156: 201–209.
98. Zhang J *et al*. Ocular pharmacokinetics of topically-applied ketoconazole solution containing hydroxypropyl beta-cyclodextrin to rabbits. *J Ocul Pharmacol Ther* 2008; 24: 501–506.
99. Reddy IK *et al*. Permeability of a soft steroid, loteprednol etabonate, through an excised rabbit cornea. *J Ocul Pharmacol Ther* 1996; 12: 159–167.
100. Siefert B, Keipert S. Influence of α-cyclodextrin and hydroxyalkylated β-cyclodextrin derivatives on the in vitro corneal uptake and permeation of aqueous pilocarpine-HCl solutions. *J Pharm Sci* 1997; 86: 716–720.
101. Freedman KA *et al*. Beta-cyclodextrins enhance bioavailability of pilocarpine. *Curr Eye Res* 1993; 12: 641–647.
102. Keipert S *et al*. Interactions between cyclodextrins and pilocarpine – as an example of a hydrophilic drug. *Int J Pharm* 1996; 142: 153–162.
103. Järvinen K *et al*. The effect of modified β-cyclodextrin, SBE4-β-CD, on the aqueous solubility and ocular absorption of pilocarpine. *Curr Eye Res* 1994; 13: 891–905.
104. Wheeler LA. The use of inclusion complexes of prostaglandins with cyclodextrins in the treatment of ocular hypertension. 3221991: Eur. Pat. #O 435 682 A3.
105. Cappello B *et al*. Formulation and preliminary in vivo testing of rufloxacin-cyclodextrin ophthalmic solutions. *J Incl Phenom Macroc Chem* 2002; 44: 173–176.
106. Siefert B *et al*. Influence of cyclodextrins on the in vitro permeability and in vivo ocular distribution of thalidomide. *J Ocul Pharmacol Ther* 1999; 15: 429–438.
107. Green K, Kearse EC. Ocular penetration of topical ?9-tetrahydrocannabinol from rabbit corneal or cul-de-sac application site. *Curr Eye Res* 2000; 21: 566–570.
108. Kearse EC, Green K. Effect of vehicle upon *in vitro* transcorneal permeability and intracorneal content of Δ^9-tetrahydrocannabinol. *Curr Eye Res* 2000; 20: 496–501.
109. Loftsson T, Järvinen T. Cyclodextrins in ophthalmic drug delivery. *Adv Drug Deliv Rev* 1999; 36: 59–79.
110. Loftsson T *et al*. Topical drug delivery to the posterior segment of the eye: anatomical and physiological considerations. *Pharmazie* 2008; 63: 171–179.
111. Loftsson T, Stefánsson E. Effect of cyclodextrins on topical drug delivery to the eye. *Drug Dev Ind Pharm* 1997; 23: 473–481.
112. Järvinen T *et al*. Sulfobutyl ether β-cyclodextrin (SBE-β-CyD) in eye drops improves the tolerability of a topically applied pilocarpine prodrug in rabbits. *J Ocul Pharmacol Ther* 1995; 11: 95–106.
113. Jarho P *et al*. Modified β-cyclodextrin (SBE7-β-CyD) with viscous vehicle improves the ocular delivery and tolerability of pilocarpine prodrug in rabbits. *J Pharm Pharmacol* 1996; 48: 264–270.
114. Suhonen P *et al*. Ocular absorption and irritation of pilocarpine prodrug is modified with buffer, polymer, and cyclodextrin in the eyedrop. *Pharmaceutical Res* 1995; 12: 529–533.
115. Chavanpatil MD, Vavia PR. The influence of absorption enhancers on nasal absorption of acyclovir. *Eur J Pharm Biopharm* 2004; 57: 483–487.
116. Hermens WAJJ *et al*. Nasal absorption enhancement of 17β-estradiol by dimethyl-β-cyclodextrin in rabbits and rats. *Pharm Res* 1990; 7: 500–503.
117. Irie T *et al*. Enhancing effects of cyclodextrins on nasal absorption of insulin in rats. *Int J Pharm* 1992; 84: 129–139.
118. Loftsson T *et al*. Cyclodextrin solubilization of benzodiazepines: formulation of midazolam nasal spray. *Int J Pharm* 2001; 212: 29–40.
119. Dale O *et al*. Intranasal midazolam: a comparison of two delivery devices in human volunteers. *J Pharm Pharmacol* 2006; 58: 1311–1318.
120. Gu F-G *et al*. Preparation of prostaglandin E1-hydroxypropyl-β-cyclodextrin complex and its nasal delivery in rats. *Int J Pharm* 2005; 290: 101–108.
121. Brown GA *et al*. Acute hormonal response to sublingual androstenediol in young men. *J Appl Physiol* 2002; 92: 142–146.
122. Figueiras A *et al*. In vitro evaluation of natural and methylated cyclodextrins as buccal permeation enhancing system for omeprazole delivery. *Eur J Pharm Biopharm* 2009; 71: 339–345.
123. Jug M *et al*. Novel cyclodextrin-based film formulation intended for buccal delivery of atenolol. *Drug Dev Ind Pharm* 2009; 35: 796–807.
124. Mannila J *et al*. Precipitation complexation method produces cannabidiol/β-cyclodextrin inclusion complex suitable for sublingual administration of cannabidiol. *J Pharm Sci* 2007; 96: 312–319.

125. Mannila J *et al*. Cyclodextrins and chitosan derivatives in sublingual delivery of low solubility peptides: a study using cyclosporin A, alpha-cyclodextrin and quaternary chitosan N-betainate. *Int J Pharm* 2010; 381: 19–24.
126. Yoo SD *et al*. Increased bioavailability of clomipramine after sublingual administration in rats. *J Pharm Sci* 1999; 88: 1119–1121.
127. Badawy SIF *et al*. Bioavailability of danazol-hydroxypropyl-β-cyclodextrin complex by different routes of administration. *Int J Pharm* 1996; 145: 137–143.
128. Jain AC *et al*. Development and in vivo evaluation of buccal tablets prepared using danazol-sulfobutylether 7 β-cyclodextrin (SBE 7) complexes. *J Pharm Sci* 2002; 91: 1659–1668.
129. Hoon TJ *et al*. Bioequivalence of 17β-estradiol hydroxypropyl-β-cyclodextrin complex in postmenopausal women. *J Clin Pharmacol* 1993; 33: 1116–1121.
130. Fridriksdóttir H *et al*. Design and in vivo testing of 17β-estradiol-HPβCD sublingual tablets. *Pharmazie* 1996; 51: 39–42.
131. Loftsson T *et al*. Sublingual delivery of 17β-estradiol from cyclodextrin containing tablets. *Pharmazie* 2003; 58: 358–359.
132. Mura P *et al*. Development of mucoadhesive films for buccal administration of flufenamic acid: effect of cyclodextrin complexation. *J Pharm Sci* 2010; 99: 3019–3029.
133. Mannila J *et al*. Sublingual administration of Δ^9-tetrahydrocannabinol/β-cyclodextrin complex increases the bioavailability of Δ^9-tetrahydrocannabinol in rabbits. *Life Sci* 2006; 78: 1911–1914.
134. Cabral-Marques H, Almeida R. Optimization of spray-drying process variables for dry powder inhalation (DPI) formulations of corticosteroid/cyclodextrin inclusion complexes. *Eur J Pharm Biopharm* 2009; 73: 121–129.
135. Kinnarinen T *et al*. Pulmonary deposition of a budesonide/γ-cyclodextrin complex in vitro. *J Controlled Release* 2003; 90: 197–205.
136. Matilainen L *et al*. In vitro evaluation of cyclodextrin complexation on pulmonary deposition of a peptide, cyclosporin A. *Int J Pharm* 2006; 318: 41–48.
137. Yang W *et al*. In vitro characterization and pharmacokinetics in mice following pulmonary delivery of itraconazole as cyclodextrin solubilized solution. *Eur J Pharm Sci* 2010; 39: 336–347.
138. Sato T *et al*. A novel administration route of edaravone – II: mucosal absorption of edaravone from edaravone/hydroxypropyl-β-cyclodextrin complex solution including L-cysteine and sodium hydrogen sulfite. *Pharmacology* 2010; 85: 88–94.
139. Kim JK *et al*. Thermo-reversible flurbiprofen liquid suppository with HP-β-CD as a solubility enhancer: improvement of rectal bioavailability. *J Incl Phenom Macroc Chem* 2009; 64: 265–272.
140. Francois M *et al*. A mucoadhesive, cyclodextrin-based vaginal cream formulation of itraconazole. *AAPS PharmSci* 2003; 5: 1. http://www.aapsj.org/view.asp?art=ps050105
141. Cevher E *et al*. Preparation and characterisation of natamycin: γ-cyclodextrin inclusion complex and its evaluation in vaginal mucoadhesive formulations. *J Pharm Sci* 2008; 97: 4319–4335.
142. Marttin E *et al*. Efficiency, safety and mechanism of cyclodextrins as absorption enhancers in nasal delivery of peptide and protein drugs. *J Drug Target* 1998; 6: 17–36.
143. Merkus FWHM *et al*. Cyclodextrin in nasal drug delivery. *Adv Drug Deliv Rev* 1999; 36: 41–57.
144. Kublik H *et al*. Nasal absorption of 17β-estradiol from different cyclodextrin inclusion formulations in sheep. *Eur J Pharm Biopharm* 1996; 42: 320–324.
145. Richter T, Keipert S. In vitro permeation studies comparing bovine nasal mucosa, porcine cornea and artificial membrane: androstenedione in microemulsions and their components. *Eur J Pharm Biopharm* 2004; 58: 137–143.
146. Cabral Marques HM *et al*. Studies of cyclodextrin inclusion complexes. III. The pulmonary absorption of β-, DM-β- and HP-β-cyclodextrins in rabbits. *Int J Pharm* 1991; 77: 297–302.
147. Hussain A *et al*. Absorption enhancers in pulmonary protein delivery. *J Controlled Release* 2004; 94: 15–24.
148. Evrard B *et al*. Cyclodextrins as a potential carrier in drug nebulization. *J Controlled Release* 2004; 96: 403–410.
149. Luengo J *et al*. Preliminary pharmacokinetic study of different preparations of acyclovir with β-cyclodextrin. *J Pharm Sci* 2002; 91: 2593–2598.
150. Evrard B *et al*. Oral bioavailability in sheep of albendazole from a suspension and from a solution containing hydroxypropyl-β-cyclodextrin. *J Controlled Release* 2002; 85: 45–50.
151. García JJ *et al*. Bioavailability and efficiacy characteristics of two different oral liquid formulations of albendazole. *Int J Pharm* 2003; 250: 351–358.
152. Castillo JA *et al*. Preparation and characterization of albendazole β-cyclodextrin complexes. *Drug Dev Ind Pharm* 1999; 25: 1241–1248.
153. Kalaiselvan R *et al*. Enhancement of bioavailability and anthelmintic efficacy of albendazole by solid dispersion and cyclodextrin complexation techniques. *Pharmazie* 2007; 62: 604–607.
154. Ren K *et al*. Physicochemical characteristics and oral bioavailability of andrographolide complexed with hydroxypropyl-β-cyclodextrin. *Pharmazie* 2009; 64: 515–520.
155. Wong JW, Yuen KH. Improved oral bioavailability of artemisinin through inclusion complexation with β- and γ-cyclodextrins. *Int J Pharm* 2001; 227: 177–185.
156. El-Gindy GA *et al*. Preparation, pharmacokinetic and pharmacodynamic evaluation of carbamazepine inclusion complexes with cyclodextrins. *STP Pharma Sci* 2002; 12: 369–378.
157. Brewster ME *et al*. Intravenous and oral pharmacokinetic evaluation of a 2-hydroxypropyl-β-cyclodextrin-based formulation of carbamazepine in the dog: comparison with commercially available tablets and suspensions. *J Pharm Sci* 1997; 86: 335–339.
158. Betlach CJ *et al*. Oral pharmacokinetics of carbamazepine in dogs from commercial tablets and a cyclodextrin complex. *J Pharm Sci* 1993; 82: 1058–1060.
159. Choudhury S, Nelson KF. Improvement of oral bioavailability of carbamazepine by inclusion in 2-hydroxypropyl-β-cyclodextrin. *Int J Pharm* 1992; 85: 175–180.
160. Koester LS *et al*. Bioavailability of carbamazepine:β-cyclodextrin complex in beagle dogs from hydroxypropylmethylcellulose matrix tablets. *Eur J Pharm Sci* 2004; 22: 201–207.
161. Hirayama F *et al*. Crystallization and polymorphic transition behavior of chloramphemicol palmitate in 2-hydroxypropyl-β-cyclodextrin matrix. *Eur J Pharm Biopharm* 1997; 5: 23–30.
162. Patel SG, Rajput SJ. Enhancement of oral bioavailability of cilostazol by forming its inclusion complexes. *AAPS PharmSciTech* 2009; 10: 660–669.
163. Tokumura T *et al*. Enhancement of bioavailability of cinnarizine from its β-cyclodextrin complex on oral administration with DL-phenylalanine as a competing agent. *J Pharm Sci* 1985; 74: 496–497.
164. Tokumura T *et al*. Enhancement of bioavailability of cinnarizine from its β-cyclodextrin complex on oral administration with DL-phenylalanine as a competing agent. *J Pharm Sci* 1986; 75: 391–394.

165. Järvinen T *et al*. β-Cyclodextrin derivatives, SBE4-β-CD and HP-β-CD, increase the oral bioavailability of cinnarizine in beagle dogs. *J Pharm Sci* 1995; 84: 295–299.
166. Miyake K *et al*. Enhanced absorption of cyclosporin A by complexation with dimethyl-β-cyclodextrin in bile duct-cannulated and -noncannulated rats. *Biol Pharm Bull* 1999; 22: 66–72.
167. Miyake K *et al*. Improved solubility and oral bioavailability of cyclosporin A by hydrophilic cyclodextrin complexation. In: Torres Labandeira JJ, Vila-Jato JL, eds. *Proceedings of the Ninth International Symposium on Cyclodextrins*. Dordrecht: Kluwer Academic Publishers, 1999: 293–296.
168. Badawy SIF *et al*. Characterization and bioavailability of danazol-hydroxypropyl-β-cyclodextrin coprecipitates. *Int J Pharm* 1996; 128: 45–54.
169. Liversidge GG, Cundy KC. Particle-size reduction for improvement of oral bioavailability of hydrophobic drugs. 1. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. *Int J Pharm* 1995; 125: 91–97.
170. Corvi Mora P *et al*. Enhancement of dehydroepianhydrosterone solubility and bioavailability by ternary complexation with a-cyclodextrin and glycine. *J Pharm Sci* 2003; 92: 2177–2184.
171. Uekama K *et al*. Improvement of the oral bioavailability of digitalis glycosides by cyclodextrin complexation. *J Pharm Sci* 1983; 72: 1338–1341.
172. Le Corre P *et al*. Influence of hydroxypropyl-β-cyclodextrin and dimethyl-β-cyclodextrin on diphenhydramine intestinal absorption in a rat in situ model. *Int J Pharm* 1998; 169: 221–228.
173. Ricevuti G *et al*. Pharmacokinetics of dipyridamole-β-cyclodextrin complex in healthy volunteers after single and multiple doses. *Eur J Drug Metab Pharmacokinet* 1991; 16: 197–201.
174. Stracciari GL *et al*. Pharmacokinetics of dipyridamole-β-cyclodextrin complex in dogs. *Arch Int Pharmacodyn Ther* 1989; 300: 7–13.
175. Miyaji T *et al*. Improvement of oral bioavailability of fenbufen by cyclodextrin complexation. *Acta Pharm Nord* 1992; 4: 17–22.
176. Géczy J *et al*. The inclusion of fluoxitine into γ-cyclodextrin increases its bioavailability: bahavioural, electrophysiological and pharmacokinetic studies. *Psychopharmacologia* 2000; 151: 328–334.
177. Tokumura T *et al*. Improvement of oral bioavailability of flurbiprofen from flurbiprofen/β-cyclodextrin inclusion complex by action of cinnarizine. *Eur J Pharm Biopharm* 2009; 73: 202–204.
178. Savolainen J *et al*. Coadministration of a water-soluble polymer increases the usefulness of cyclodextrins in solid dosage forms. *Pharm Res* 1998; 15: 1696–1701.
179. Jayachandra Babu R, Pandit JK. Enhancement of dissolution rate and hypoglycemic activity of glibenclamide with β-cyclodextrin. *STP Pharma Sci* 1995; 5: 196–201.
180. Aggarwal S *et al*. Studies on solubility and hypoglycemic activity of gliclazide β-cyclodextrin-hydroxypropylmethylcellulose complexes. *Pharmazie* 2002; 57: 191–193.
181. Sridevi S *et al*. Enhancement of dissolution and oral bioavailability of gliquidone with hydroxy propyl-β-cyclodextrin. *Pharmazie* 2003; 58: 807–810.
182. Uekama K *et al*. Improvement of dissolution and absorption characteristics of indomethacin by water-soluble a-cyclodextrin-epichlorohydrin polymer. *Acta Pharm Suec* 1987; 24: 27–36.
183. Jambhekar S *et al*. The physicochemical characteristics and bioavailability of indomethacin from β-cyclodextrin, hydroxyethyl-β-cyclodextrin, and hydroxypropyl-β-cyclodextrin complexes. *Int J Pharm* 2004; 270: 149–166.
184. Ahn HJ *et al*. Effects of cyclodextrin derivatives on bioavailability of ketoprofen. *Drug Dev Ind Pharm* 1997; 23: 397–401.
185. Emara LH *et al*. Improving the dissolution and bioavailability of nifedipine using solid dispersions and solubilizers. *Drug Dev Ind Pharm* 2002; 28: 795–807.
186. Uekama K *et al*. Inhibitory effect of 2-hydroxypropyl-β-cyclodextrin on crystal-growth of nifedipine during storage – superior dissolution and oral bioavailability compared with polyvinylpyrrolidone K-30. *J Pharm Pharmacol* 1992; 44: 73–78.
187. Wang Z *et al*. In-vivo and in-vitro evaluations of modified-release oral dosage form of nifedipine by hybridization of hydoxypropyl-β-cyclodextrin and hydroxypropylcellulose in dogs. *J Pharm Pharmacol* 1994; 46: 505–507.
188. Chowdary KPR, Kamalakara RG. Controlled release of nifedipine from mucoadhesive tablets of its inclusion complexes with β-cyclodextrin. *Pharmazie* 2003; 58: 721–724.
189. Uekama K *et al*. Improvement of dissolution and absorption characteristics of phenytoin by a water-soluble β-cyclodextrin-epichlorohydrin polymer. *Int J Pharm* 1985; 23: 35–42.
190. Tanino T *et al*. Effect of sugar-modified β-cyclodextrins on dissolution and absorption characteristics of phenytoin. *Biol Pharm Bull* 1999; 22: 298–304.
191. Savolainen J *et al*. Improved dissolution and bioavailability of phenytoin by sulfobutylether-β-cyclodextrin ($(SBE)_{7m}$-β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) complexation. *Int J Pharm* 1998; 165: 69–78.
192. Elkheshen SA *et al*. Inclusion complexes of piroxicam with β-cyclodextrin derivatives in comparison with the natural β-cyclodextrin – 2nd communication: in vitro and in vivo drug availability. *Pharm Ind* 2002; 64: 708–715.
193. Woodcock BG *et al*. Supermolecular inclusion of piroxicam with β-cyclodextrin: pharmacokinetic properties in man. *Eur J Rheumatol Inflamm* 1993; 12: 12–28.
194. Kimura E *et al*. Pharmacokinetic profile of piroxicam β-cyclodextrin, in rat plasma and lymph. *Gen Pharmacol* 1997; 28: 695–698.
195. Deroubaix X *et al*. Oral bioavailability of CHF1194, an inclusion complex of piroxicam and b-cyclodextrin, in healthy-subjects under single-dose and steady-state conditions. *Eur J Clin Pharmacol* 1995; 47: 531–536.
196. McEwen J. Clinical pharmacology of piroxicam-β-cyclodextrin. Implications for innovative patient care. *Clin Drug Investig* 2000; 19(Suppl. 2): 27–31.
197. Wempe MF *et al*. Pharmacokinetics of raloxifene in male Wistar–Hannover rats: influence of complexation with hydroxybutenyl-beta-cyclodextrin. *Int J Pharm* 2008; 346: 25–37.
198. Chavanpatil M *et al*. Enhancement of oral bioavailability of rofecoxib using β-cyclodextrin. *J Incl Phenom Macroc Chem* 2002; 44: 145–149.
199. Kaukonen AM *et al*. Water-soluble β-cyclodextrins in pediatric oral solutions of spironolactone: preclinical evaluation of spironolactone bioavailability from solutions of β-cyclodextrin derivatives in rats. *J Pharm Pharmacol* 1998; 50: 611–619.
200. Soliman OAE *et al*. Amorphous spirolactone-hydroxypropylated cyclodextrin complexes with superior dissolution and oral bioavailability. *Int J Pharm* 1997; 149: 73–83.
201. Seo H *et al*. Enhancement of oral bioavailability of spironolactone by β- and γ-cyclodextrin complexations. *Chem Pharm Bull* 1983; 31: 286–291.

202. Arima H *et al*. Comparative studies of the enhancing effects of cyclodextrins on the solubility and oral bioavailability of tacrolimus in rats. *J Pharm Sci* 2001; 90: 690–701.
203. Veiga F *et al*. Oral bioavailability and hypoglycaemic activity of tolbutamide/cyclodextrin inclusion complexes. *Int J Pharm* 2000; 202: 165–171.
204. Kimura K *et al*. Effects of aging on crystallization, dissolution and absorption characteristics of amorphous tolbutamide-2-hydroxypropyl-β-cyclodextrin complex. *Chem Pharm Bull* 2000; 48: 646–650.
205. Moffat AC *et al*., ed. *Clarke's Analysis of Drugs and Poisons*, 3rd edn. Vol. 2. London: Pharmaceutical Press, 2004.
206. Carrier RL *et al*. The ultility of cyclodextrins for enhancing oral bioavailability. *J Controlled Release* 2007; 123: 78–99.
207. Dahan A *et al*. The solubility–permeability interplay in using cyclodextrins as pharmaceutical solubilizers: Mechanistic modeling and application to progesterone. *J Pharm Sci* 2010; 99: 2739–2749.
208. Loftsson T. Cyclodextrins and the biopharmaceutics classification system of drugs. *J Incl Phenom Macroc Chem* 2002; 44: 63–67.
209. Loftsson T *et al*. Role of cyclodextrins in improving oral drug delivery. *Am J Drug Deliv* 2004; 2: 261–275.
210. Karlsson JP, Artursson P. A method for the determination of cellular permeability coefficients and aqueous boundary layer thickness in monolayers of intestinal epithelia (Caco-2) cells grown in permeable filter chambers. *Int J Pharm* 1991; 71: 55–64.
211. Youdim KA *et al*. *In vitro* trans-monolayer permeability calculations: often forgotten assumptions. *Drug Discov Today* 2003; 8: 997–1003.
212. Zuo Z *et al*. Flutamide – hydroxypropyl-β-cyclodextrin complex: formulation, physical characterization, and absorption using the caco-2 in vitro model. *J Pharm Pharm Sci* 2000; 3: 220–227.
213. Yavuz B *et al*. Alternative oral exemestane formulation: improved dissolution and permeation. *Int J Pharm* 2010; 398: 137–145.
214. Kuang H- *et al*. Transport research of ibuprofen/HP-β-CD system in the Caco-2 monolayer model. *Zhongguo Xinyao Zazhi* 2008; 34: 1037–1041.
215. Lambert D *et al*. Methyl-β-cyclodextrin increases permeability of caco-2 cell monolayers by displacing specific claudins from cholesterol rich domains associated with tight junctions. *Cell Physiol Biochem* 2007; 20: 495–506.
216. Ruell JA *et al*. PAMPA – a drug absorption in vitro model 5. Unstirred water layer in iso-pH mapping assay and pKa$_{flux}$-optimized design (pOH-PAMPA). *Eur J Pharm Sci* 2003; 20: 393–402.
217. Avdeef A *et al*. PAMPA – a drug absorption in vitro model 11. Matching the in vivo unstirred water layer thickness by individual-well stirring in microtitre plates. *Eur J Pharm Sci* 2004; 22: 365–374.
218. Avdeef A *et al*. PAMPA – critical factors for better predictions of absorption. *J Pharm Sci* 2007; 96: 2893–2909.
219. Brewster ME *et al*. Effect of the unstirred water layer on permeability enhancement by hydrophilic cyclodextrins. *Int J Pharm* 2007; 342: 250–253.
220. Giacomini KM *et al*. Membrane transporters in drug development. *Nat Rev Drug Discov* 2010; 9: 215–236.
221. Bansil R, Turner BS. Mucin structure, aggregation, physiological functions and biomedical applications. *Curr Opin Colloid Interface Sci* 2006; 11: 164–170.
222. Lennernäs H. Human intestinal permeability. *J Pharm Sci* 1998; 87: 403–410.
223. Norris DA *et al*. The effect of physical barriers and properties on the oral absorption of particulates. *Adv Drug Deliv Rev* 1998; 34: 135–154.
224. Khanvilkar K *et al*. Drug transfer through mucus. *Adv Drug Deliv Rev* 2001; 48: 173–193.
225. Lee SP, Nicholls JF. Diffusion of charged ions in mucus gel: effect of net charge. *Biorheology* 1987; 24: 565–569.
226. Olmsted SS *et al*. Diffusion of macromolecules and virus-like particles in human cervical mucus. *Biophys J* 2001; 81: 1930–1937.
227. Cone RA. Barrier properties of mucus. *Adv Drug Deliv Rev* 2009; 61: 75–85.
228. Cone R. Mucus. In: Ogra PL *et al*, ed. *Mucosal Immunology*. San Diego, CA: Academic Press, 1999: 43–63.
229. Behrens I *et al*. Transport of lipophilic drug molecules in new mucus-secreting cell culture model based on HT29-MTX cells. *Pharm Res* 2001; 18: 1138–1145.
230. Smith EW, Maibach HI, eds. *Percutaneous Penetration Enhancers*. Boca Raton, FL: CRC Press, 1995.
231. Whitehead K, Mitragotri S. Mechanistic analysis of chemical permeation enhancers for oral drug delivery. *Pharm Res* 2008; 25: 1412–1419.
232. Rai V *et al*. A transdermal review on permeation of drug formulations, modifier compounds and delivery methods. *J Drug Deliv Sci Technol* 2010; 20: 75–87.
233. Brouwers J *et al*. Supersaturating drug delivery systems: the answer to solubility-limited oral bioavailability? *J Pharm Sci* 2009; 98: 2549–2572.
234. Yamagata T *et al*. Improvement of the oral drug absorption of topotecan through the inhibition of intestinal xenobiotic efflux transporter, breast cancer resistance protein, by excipients. *Drug Metab Dispos* 2007; 35: 1142–1148.
235. Higuchi T. Physical chemical analysis of percutaneous absorption process from creams and ointments. *J Soc Cosmet Chem* 1960; 11: 85–97.
236. Idson B. Biophysical factors in skin penetration. *J Soc Cosmet Chem* 1971; 22: 615–634.
237. Másson M *et al*. Investigation of drug-cyclodextrin complexes by a phase-distribution method: some theoretical and practical considerations. *Chem Pharm Bull* 2005; 53: 958–964.
238. Pohl P *et al*. The size of the unstirred layer as a function of the solute diffusion coefficient. *Biophys J* 1998; 75: 1403–1409.
239. Zwolinski BJ *et al*. Diffusion and membrane permeability. I. *J Phys Colloid Chem* 1949; 53: 1426–1453.
240. Flynn GL *et al*. Total mathematical resolution of diffusion layer control of barrier flux. *J Pharm Sci* 1972; 61: 312–314.
241. Flynn GL, Yalkowsky SH. Correlation and prediction of mass transport across membranes I: influence of alkyl chain length on flux-determining properties of barrier and diffusant. *J Pharm Sci* 1972; 61: 838–852.
242. Loftsson T *et al*. 2-Hydroxypropyl-β-cyclodextrin in topical carbonic anhydrase inhibitor formations. *Eur J Pharm Sci* 1994; 1: 175–180.