International Journal of Pharmaceutics 379 (2009) 32–40

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

CDs as solubilizers: Effects of excipients and competing drugs

Phatsawee Jansook, Thorsteinn Loftsson*

Faculty of Pharmaceutical Sciences, University of Iceland, Hofsvallagata 53, IS-107 Reykjavik, Iceland

ARTICLE INFO

Article history:
Received 24 February 2009
Received in revised form 2 June 2009
Accepted 3 June 2009
Available online 12 June 2009

Keywords:
Cyclodextrins
Formulation
Solubility
Aggregates
Permeation
Availability

ABSTRACT

In recent years cyclodextrins (CDs) have been acknowledged by the pharmaceutical industry as very useful enabling excipients for solubilization and stabilization of drugs in aqueous formulations. Their effect is however strongly influenced by other commonly used excipients. The purpose of this investigation was to examine the effects of excipients and drug combinations on the effects of CD solubilization of drugs and drug availability. The model drug was dexamethasone, the competing drugs tested were hydrocortisone, indomethacin and amphotericin B, and the sample CDs were γ-cyclodextrin (γCD) and 2-hydroxypropyl-γ-cyclodextrin (HPγCD). Benzalkonium chloride and hydroxypropyl methylcellulose enhance the solubilizing effect of the CDs whereas in general EDTA decreased the effect. The effect of second drug present in the aqueous formulation did depend on the affinity of that drug for the CD. Drugs which readily formed complexes with the CDs (e.g. hydrocortisone) decreased their ability to solubilize dexamethasone. Drugs that have little affinity for CDs (e.g. amphotericin B) did in some cases improve the CD solubilization of dexamethasone. Flux diagrams obtained through semi-permeable cellophane membrane indicated that drug/CD complexes self-assemble to form aggregates, especially at CD concentrations above 5% (w/v). This aggregate formation was affected by the excipients and did influence drug availability from the formulations.

1. Introduction

Cyclodextrins (CDs) are cyclic torus-shaped molecules, consisting of 6–8 D-(+)-glucopyranose units with hydrophilic outer surface and lipophilic central cavity. During the past two decades CDs have received growing attention, mainly due to their ability to increase aqueous solubility and stability of poorly water-soluble drugs through formation of inclusion complexes (Brewster and Loftsson, 2007; Loftsson and Duchêne, 2007). Furthermore CDs can act as permeation enhancers by keeping hydrophobic drug molecules in solution and deliver them to the surface of a biological membrane, thus leading to improve transepithelial permeation and bioavailability of drug (Loftsson et al., 2007). Pharmaceutical excipients that are present in a given drug formulation can enhance or decrease the solubilizing effect of CDs (Loftsson and Brewster, 1996; Loftsson et al., 1999). Polymers can enhance the CD complexation of drugs and they can enhance the drug permeation through biological membranes, possibly through formation of ternary complexes or co-complexes (Jarho et al., 1998; Kristinsson et al., 1996; Loftsson, 1998; Mura et al., 2001; Chowdary and Srinivas, 2006). Frequently dosage forms contain more than one active ingredient. Dexamethasone can, for example, be found in various combination eye drops such as eye drops containing dexamethasone, neomycin and polymyxin B, and eye drops containing dexamethasone and tobramycin. However, combination products containing CD have not been marketed.

The purpose of the present investigation is to study the influence of common pharmaceutical excipients, as well as that of competing drugs, on the CD solubilization of drugs that possess poor solubility in water and their availability. Aqueous eye drop solution was used as a sample formulation with dexamethasone as a model drug. The tested competing drugs were hydrocortisone, which has similar steroidal structure as dexamethasone, indomethacin, which is a carboxylic acid fully ionized at physiologic pH, and amphotericin B, which is a water-insoluble polyene antibiotic that has low affinity for the CD central cavity. The sample CDs were γ-cyclodextrin (γCD), which has limited solubility in water, and its water-soluble derivative 2-hydroxypropyl-γ-cyclodextrin (HPγCD). Previous studies have shown that mixtures of γCD and HPγCD can be more potent solubilizers than the individual CDs (Jansook and Loftsson, 2008). Thus, mixtures of γCD and HPγCD were also included in this study. The physicochemical properties of the excipients and sample compounds are shown in Table 1.

2. Materials and methods

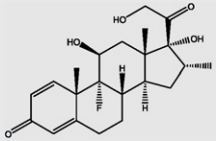
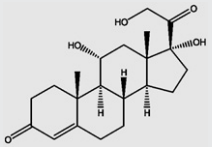
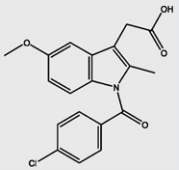
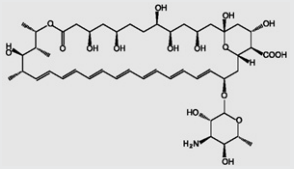
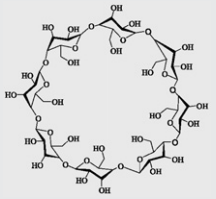
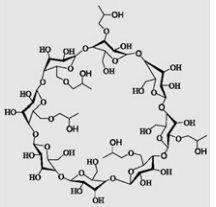
2.1. Materials

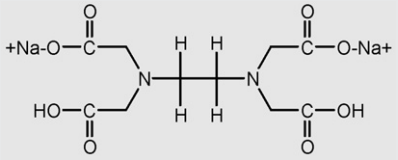
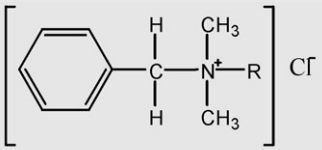
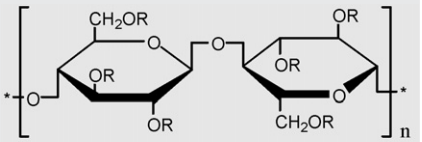
Dexamethasone (Dx) was purchased from Fagron group (Amsterdam, Netherlands), hydrocortisone (HC) from ICN Biomedicals

* Corresponding author. Tel.: +354 525 4464; fax: +354 525 4071.
E-mail address: thorstlo@hi.is (T. Loftsson).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.06.005

Table 1
Physicochemical properties of the excipients and sample compounds (Lavasanifar et al., 2002; Moffat et al., 2004; Nokhodchi et al., 2005; Brewster and Loftsson, 2007).

Physicochemical properties	(A)					
	Dexamethasone	Hydrocortisone	Indomethacin	Amphotericin B	γCD	HPγCD [a]
Chemical structure						
Molecular weight	392.5	362.5	357.8	924.1	1297.1	1576
Melting point (°C)[b]	270 (dec.)	214 (dec.)	158	>170 (dec.)	≥200 (dec.)	≥200 (dec.)
pK_a	–	–	4.5	5.5; 10	–	–
Octanol/water partition coefficient	1.8	1.6	−1.0 (at pH 7.4)	0.8	−12	<−10
S_0 (mg/ml) in water (at RT)	0.08	0.4	0.8 (at pH 7.2)	0.001	249	>500

Physicochemical properties	(B)		
	Edetate disodium	Benzalkonium chloride	Hydroxypropylmethylcellulose
Chemical structure		 R= mixture of alkyls: n-C_8H_{17} to n-$C_{18}H_{37}$; mainly $C_{12}H_{25}$ (dodecyl), n-$C_{14}H_{29}$ (tetradecyl), and n-$C_{16}H_{33}$ (hexadecyl).	 where R is H, CH_3, or $CH_3CH(OH)CH_2$
Molecular weight	336.2	360 (average)	10,000–1,500,000 (approximately)
Melting point (°C)[b]	252 (dec.)	≈40	190–200 (browns)
pK_a	2.0; 2.7; 6.2; 10.3	–	–
Octanol/water partition coefficient	–	9.98 for C_{12}; 32.9 for C_{14}; 82.5 for C_{16} [c]	–
S_0 (mg/ml) in water (at RT)	96	Very soluble	Varies with the viscosity

[a] Representative structure.
[b] Dec.: decomposition upon heating.
[c] Varies with the alkyl chain length of the homolo.

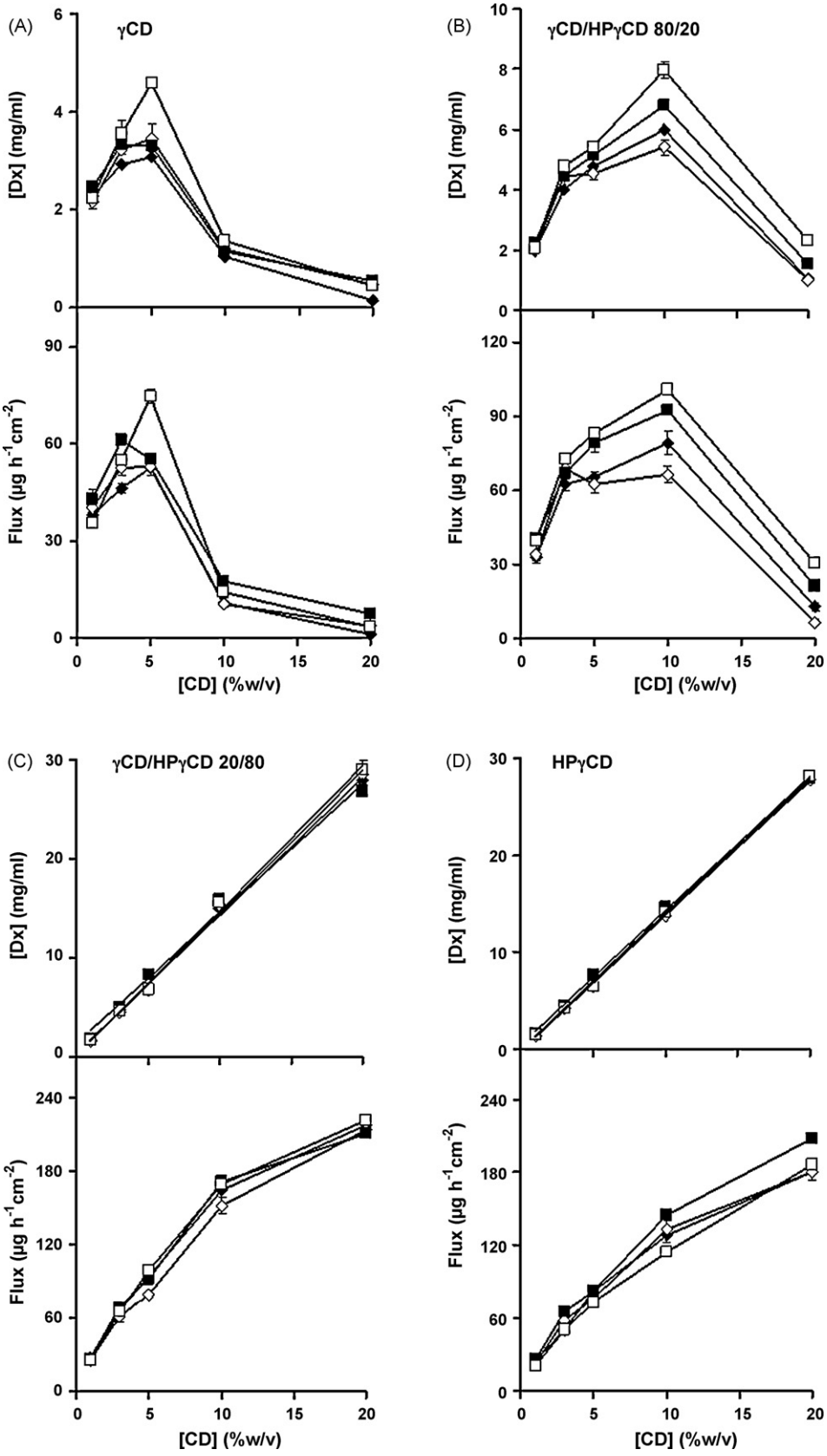


Fig. 1. The effect of additives in aqueous CD solution on the solubility and the flux of dexamethasone through semi-permeable cellophane membrane MWCO 3500; (A) γCD; (B) γCD/HPγCD (ratio 80:20); (C) γCD/HPγCD (ratio 20:80); (D) HPγCD; (♦) EDTA (0.1%, w/v); (◊) BAC (0.02%, w/v); (■) HPMC (0.1%, w/v); (□) all excipients (i.e. in the aqueous eye drop formulation).

Table 2
HPLC conditions.

Drugs	Mobile phase[a]	Flow rate (ml/min)	Wavelength (nm)	Retention time (min)
Dexamethasone	ACN:THF:water (33:1:66)	1.5	241	5.1
Dexamethasone Hydrocortisone	ACN:THF:water (33:1:66)	1.4	241 and 254	5.1 and 3.0
Dexamethasone Indomethacin	ACN:0.5% acetic acid (50:50)	1.5	240 and 240	7.2 and 1.9
Dexamethasone amphotericin B	ACN:0.25 mM EDTA (37:63)	1.0	241 and 403	4.4 and 3.1

[a] Volume ratios. ACN: acetonitrile; THF: tetrahydrofuran; acetic acid: aqueous acetic acid solution; EDTA: aqueous disodium edetate dehydrate solution.

(Aurora, OH), amphotericin B (AmB) and indomethacin (IDM) from Sigma (St. Louis, MO), γ-cyclodextrin (γCD) and 2-hydroxypropyl-γ-cyclodextrin (HPγCD) MS 0.6 (MW 1576 Da) from Wacker Chemie (Munich, Germany), disodium edetate dehydrate (EDTA) and sodium chloride (NaCl) from Merck (Darmstadt, Germany), benzalkonium chloride (BAC) and hydroxypropyl methylcellulose 4000 (HPMC) from Sigma (St. Louis, MO), semi-permeable cellophane membranes (SpectaPor®, molecular weight cut-off (MWCO) 3500) from Spectrum Europe (Breda, Netherlands). All other chemicals used were of analytical reagent grade purity. Milli-Q (Millipore, Bedford, MA) water was used for the preparation of all solutions.

2.2. Solubility determinations

Solubility of dexamethasone and in water or aqueous CD solutions was determined by heating in autoclave (121 °C for 20 min) (Loftsson and Hreinsdóttir, 2006). Excess amount of dexamethasone was added to an aqueous solution containing 0–20% (w/v) CD (pure γCD, pure HPγCD, or a mixture of γCD and HPγCD), benzalkonium chloride (0.02%, w/v), EDTA (0.1%, w/v) and/or hydroxypropyl methylcellulose (HPMC) (0.1% w/v), individual compounds or mixtures thereof. The effect of HPMC on the solubility of dexamethasone was determined in 0.10–0.75% (w/v) HPMC solutions in pure water. The suspensions formed were heated in autoclave at 121 °C for 20 min in sealed glass vials and then allowed to cool to room temperature. Then small amount of solid drug was added to the suspensions, pH adjusted to 7.4 with concentrated aqueous hydroxide solution, and the suspension allowed to equilibrate in the resealed vials at room temperature (22–23 °C) for 7 days under constant agitation. Many drugs such as indomethacin are known to exist in more than one polymorphic form and thus it is essential to add small amount of the solid drug to the test media after heating. After equilibrium was attained, the suspensions were filter through 0.45 μm membrane filters, the filtrates diluted with mobile phase and analyzed by HPLC. The phase-solubility profiles were determined according to Higuchi and Connors (1965).

Co-complexation of two different drugs was also investigated. In that case excess of both dexamethasone and a second drug (hydrocortisone, indomethacin or amphotericin B) were simultaneously added to the aqueous complexation media and the solubility of both drugs determined as previously described, except when the chemically instable amphotericin B was present then heating in an autoclave was replaced by heating in an ultrasonic bath at 60 °C for

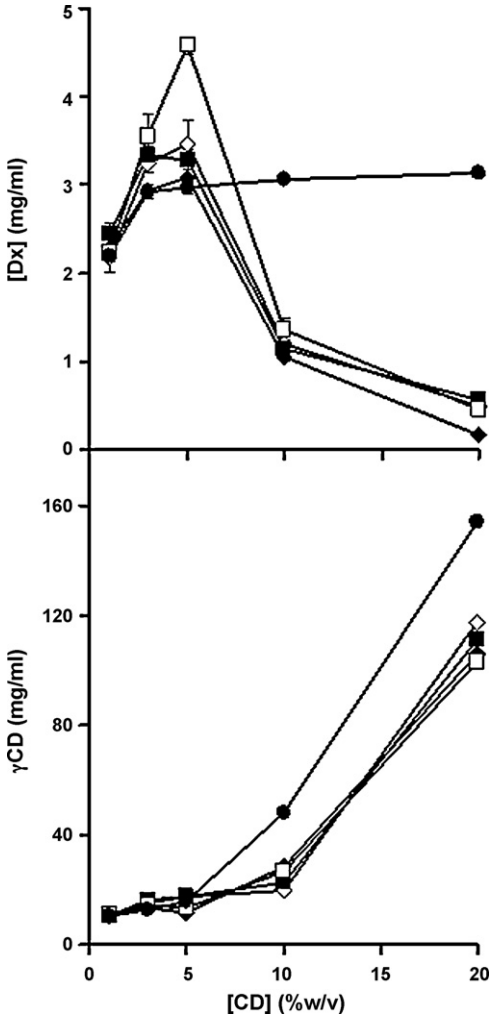


Fig. 2. The phase solubility diagrams of dexamethasone and the determined γCD content in aqueous γCD solution containing no additives and with additives; (●) no additive; (♦) EDTA (0.1% w/v); (◊) BAC (0.02%, w/v); (■) HPMC (0.1%, w/v); (□) all excipients (i.e. in the aqueous eye drop formulation). The aqueous cyclodextrin solutions were in all cases saturated with dexamethasone.

Table 3
Effect of additives on dexamethasone CE using the CE obtained in aqueous complexation medium without additives as a reference.

The additives[a]	γCD[b]		HPγCD		γCD/HPγCD			
					(80/20)[b]		(20/80)	
	CE	Ratio	CE	Ratio	CE	Ratio	CE	Ratio
No additive	0.14 ± 0.01	1.00	1.11 ± 0.05	1.00	0.62 ± 0.03	1.00	1.44 ± 0.02	1.00
EDTA	0.12 ± 0.01	0.88	1.28 ± 0.02	1.15	0.52 ± 0.03	0.85	1.16 ± 0.03	0.81
BAC	0.21 ± 0.02	1.57	1.26 ± 0.01	1.14	0.71 ± 0.04	1.16	1.23 ± 0.03	0.85
HPMC	0.17 ± 0.02	1.25	1.24 ± 0.03	1.11	0.69 ± 0.01	1.13	1.02 ± 0.01	0.71
EDTA + BAC + HPMC	0.27 ± 0.02	1.95	1.31 ± 0.02	1.18	0.87 ± 0.02	1.42	1.41 ± 0.01	0.98

[a] Concentration of additives: EDTA 0.1% (w/v); BAC 0.02% (w/v); HPMC 0.1% (w/v).
[b] Calculated from the initial slope of the B_s-type phase–solubility diagram (cyclodextrin concentration 7–23 mM).

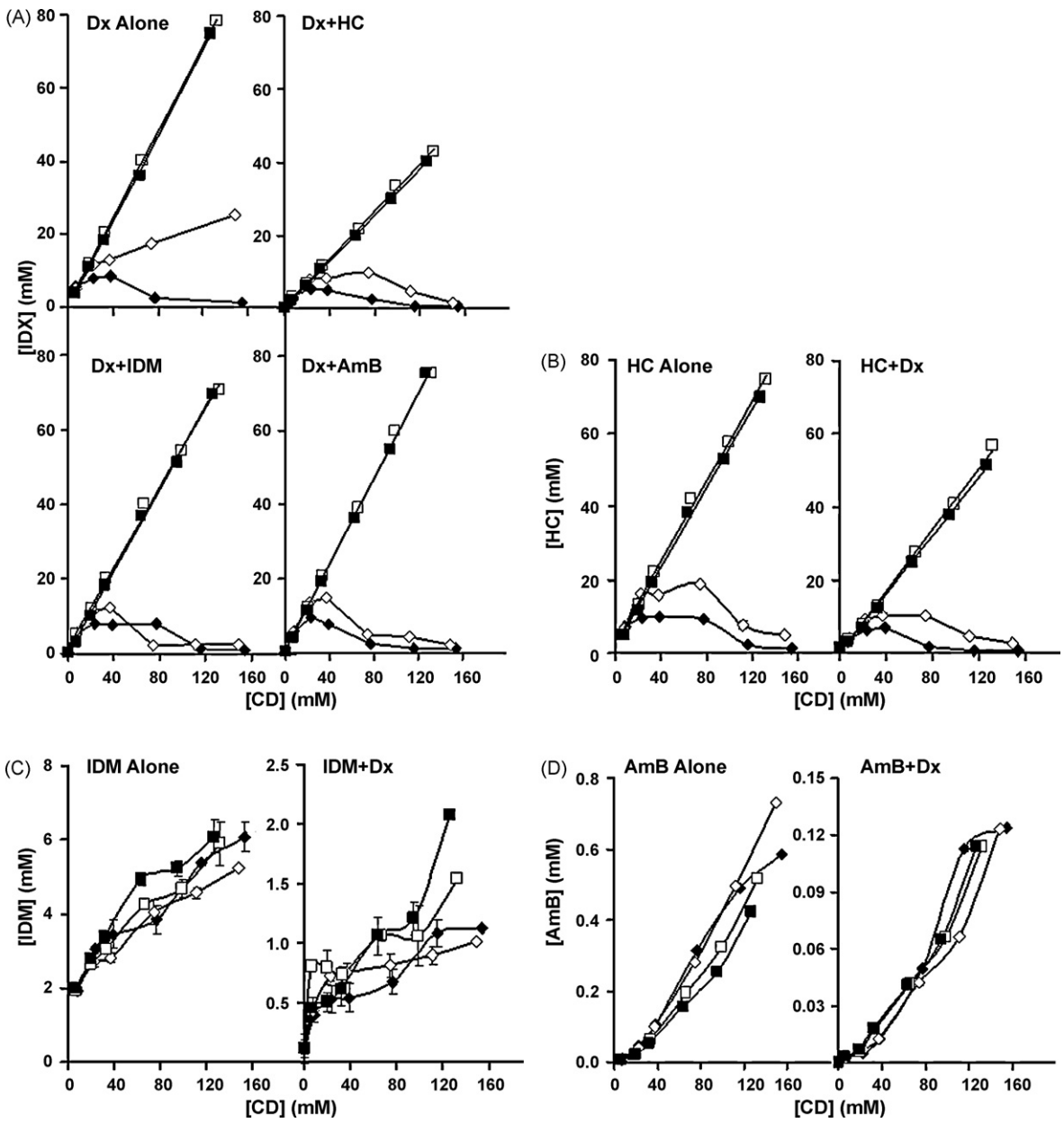


Fig. 3. The effect of a second drug in aqueous CD solution on phase solubility profiles of dexamethasone and its second drug in pure and different ratios of γCD/HPγCD mixtures in the aqueous eye drop formulation: (A) dexamethasone (DX); (B) hydrocortisone (HC); (C) indomethacin (IDM); (D) amphotericin B (AmB). γCD (♦); γCD/HPγCD ratio (80/20) (◊); (□) γCD/HPγCD ratio (20/80); (■) HPγCD.

30 min. The complexation efficiency (CE) was determined from the linear phase-solubility diagrams (plots of the total drug solubility ($[drug]_t$) versus total CD concentration ($[CD]_t$) in moles per liter) (Loftsson et al., 2005):

$$CE = \frac{Slope}{1 - Slope} = \frac{[drug/CD\ complex]}{[CD]} = K_{1:1} \cdot S_0 \quad (1)$$

where $K_{1:1}$ is the stability constant of the drug/CD 1:1 complex and S_0 is the intrinsic solubility of the drug.

2.3. Quantitative determinations

Quantitative determinations of the individual drugs were performed on a reversed-phase high performance liquid chromatographic (HPLC) component system consisting of Hewlett Packard Series 1100, consisting of a G132A binary pump with a G1379A solvent degasser, a G13658 multiple wavelength detector, a G1313A auto sampler, and Phenomenex Luna 5 μ C18 reverse-phase column (150 mm × 4.6 mm). The HPLC chromatographic conditions are shown in Table 2. Quantitative analysis of γCD content was determined by HPLC (Dionex UltiMate 3000, USA). The liquid chromatograph comprised of an UltiMate 3000 and a differential refractive index detector (Shodex RI-101, Japan) with a sensitivity of 600 μRIU. Data integration was done using CHROMELEON® software version 6.80 for LC integration. The column used was Luna NH_2 100A (10 μm, 250 mm × 4.6 mm) (Phenomenex, USA). The HPLC conditions were as follows. Mobile phase: 67% (v/v) acetonitrile in pure water; flow rate: 1 ml/min; injection volume: 20 μl; and column oven temperature: 25 °C.

2.4. Permeation studies

The permeability studies of dexamethasone, hydrocortisone, indomethacin and amphotericin B from eye drop preparations (the

donor phase) were carried out using Franz diffusion cell system (FDC 400 15FF, Vangard International, Neptune, NJ). The donor chamber and the receptor chamber were separated with a single semi-permeable cellophane membrane. The membrane was soaked overnight in the receptor phase that consisted of aqueous pH 7.4 phosphate buffer saline solution containing 5% (w/v) γCD/HPγCD (1:1 weight ratio) mixture. CD was added to the receptor phase to ensure sufficient drug solubility. The receptor phase was sonicated under vacuum to remove dissolved air before it was placed in the receptor chamber. The study was carried out at ambient temperature (22–23 °C) under continuous stirring of the receptor phase by magnetic stirring bar rotating at 300 rpm. A 150 μl sample of receptor medium was withdrawn at 30, 60, 120, 180, 240, and 360 min and replaced immediately with an equal volume of fresh receptor phase. The drug concentration in the receptor sample was determined by HPLC. The steady state flux was calculated as the slope (dq/dt) of linear section of the amount of drug in the receptor chamber (q) versus time (t) profiles, and the apparent permeability coefficient (P_{app}) was calculated from the flux (J) according to Eq. (2):

$$J = \frac{dq}{A\,dt} = P_{app}\,C_d \quad (2)$$

where A is the surface area of the mounted membrane (1.77 cm^2) and C_d is the initial concentration of the drug in the donor phase.

2.5. Aqueous eye drop sample formulations

The aqueous dexamethasone eye drop solutions were prepared by dissolving dexamethasone in 9 ml of an aqueous solution containing benzalkonium chloride (2 mg), EDTA (10 mg) and HPMC (10 mg) and various types and amounts of CD (pure γCD, pure HPγCD or mixtures of γCD/HPγCD). The amount of CD needed to solubilize given amount of dexamethasone (1.5–15 mg/ml) was determined from the phase-solubility profiles. The pH was adjusted to 7.4 with concentrated aqueous sodium hydroxide solution and the osmolality was adjusted to 260–330 mOsm/kg with NaCl. The final volume was adjusted to 10.0 ml and the solutions sterilized by heating in an autoclave (121 °C for 20 min). Before further testing the solutions were allowed to cool to room temperature and equilibrate for 7 days under constant agitation.

3. Results and discussions

Phase-solubility profiles of dexamethasone in aqueous solutions containing γCD or 80/20 mixture of γCD and HPγCD were of B_S-type, with an initial linear increase followed by a decrease in dexamethasone concentration (Fig. 1A and B), indicating that the complexes formed had limited solubility in the aqueous complexation media. In contrast, A_L type was observed in the complexation media containing pure HPγCD or 20/80 mixture of γCD and HPγCD (Fig. 1C and D), indicating formation of formation of water-soluble complexes (Higuchi and Connors, 1965). HPγCD consists of mixture of numerous structurally related isomers and thus HPγCD and its complexes do not in general form crystalline precipitates (Loftsson and Brewster, 1996). Table 3 shows the effects of the individual excipients on the CE. In general, EDTA decreases the solubilizing effect of the CDs where as the surface active BAC increases the effect. The water-soluble polymer HPMC results in a small increase in the solubility, especially when pure γCD is used as a solubilizer. The greatest increase in solubility is obtained when the eye drop formulation contains mixture EDTA, BAC and HPMC, and especially in eye drop formulations where large fraction of the drug is in the form of solid complex particles, i.e. the eye drop formulation containing γCD (CE ratio 1.95) and 80/20 mixture of γCD and HPγCD (CE ratio 1.42). Enhanced CE results in increased drug solubility in the aqueous eye drop formulation and consequent increased availability of the drug as can be seen in greater drug flux through the MWCO 3500 semipermeable cellophane membrane (Fig. 1). The flux diagrams are obtained by plotting the drug flux through the membrane as a function of the CD concentration in the donor phase which was saturated with the drug. Since the molecular weight of the individual dexamethasone/CD complexes (1690–1969 Da) are much less than the molecular weight cutoff of the membrane (3500 Da) the flux (J) should be proportional to the total amount of dissolved

Table 4
The apparent complexation constant and CE of various drugs in eye drop preparations.

Cyclodextrin	γCD/HPγCD ratio	CE (individual)	CE (combination)			
			Dexamethasone		Second drug	
			CE value	CE ratio [a]	CE value	CE ratio [b]
Dexamethasone						
γCD	–	0.26	–	–	–	–
γCD/HPγCD	80/20	0.69	–	–	–	–
γCD/HPγCD	20/80	1.26	–	–	–	–
HPγCD	–	1.07	–	–	–	–
Hydrocortisone						
γCD	–	0.31	0.07	0.28	0.11	0.35
γCD/HPγCD	80/20	1.90	0.24	0.35	0.32	0.17
γCD/HPγCD	20/80	1.61	0.48	0.38	0.74	0.46
HPγCD	–	1.21	0.45	0.42	0.68	0.56
Indomethacin						
γCD	–	0.09	0.18	0.70	0.005	0.06
γCD/HPγCD	80/20	0.06	0.69	1.00	0.003	0.05
γCD/HPγCD	20/80	0.07	1.11	0.88	0.006	0.09
HPγCD	–	0.07	1.21	1.13	0.006	0.08
Amphotericin B						
γCD	–	0.07	0.30	1.17	0.0007	0.01
γCD/HPγCD	80/20	0.07	1.71	1.76	0.0006	0.01
γCD/HPγCD	20/80	0.05	1.32	1.05	0.0007	0.01
HPγCD	–	0.04	1.40	1.31	0.0007	0.02

[a] CE of dexamethasone when the second drug is also present in the complexation medium/CE of dexamethasone in the aqueous complexation medium when no other drug is present.

[b] CE of the drug in presence of dexamethasone/CE of the drug when no dexamethasone is present.

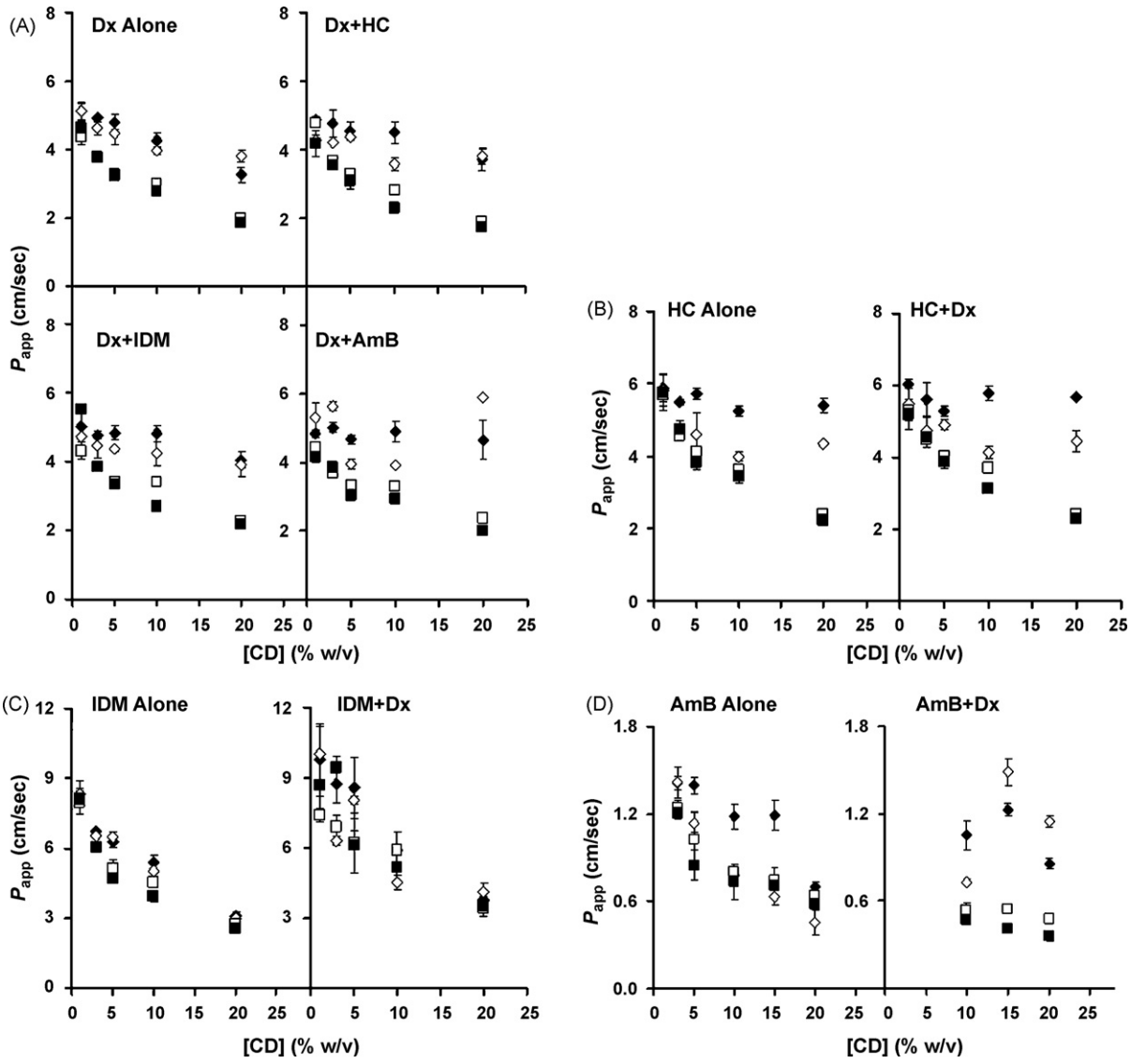


Fig. 4. The effect of a second drug in aqueous CD solution on the P_{app} of dexamethasone and its second drug in pure and different ratios of γCD/HPγCD mixtures in the aqueous eye drop formulation through semi-permeable cellophane membrane MWCO 3500: (A) dexamethasone (Dx); (B) hydrocortisone (HC); (C) indomethacin(IDM); (D) amphotericin B (AmB). (♦) γCD; (◊) γCD/HPγCD ratio (80/20); (□) γCD/HPγCD ratio (20/80); (■) HPγCD.

drug (C_d) in the donor phase (Eq. (2)). This is indeed observed in Fig. 1A and B. However, the flux diagrams obtained from donor phases containing either HPγCD or γCD/HPγCD 20/80 mixture with other excipients (Fig. 1C and D), showed a negative deviation from linearity, while their phase-solubility profiles are linear. The main reason of such negative deviations is self-assemble of the drug/CD complexes to form water-soluble aggregates that are too large to be able to permeate the membranes but too small to display static light scattering and thus the aqueous solutions appear clear to the naked eye (Loftsson et al., 2002, 2004; Jansook et al., in press).

Both BAC and HPMC enhance dexamethasone solubility at CD concentrations below 5% (w/v) but decrease the solubility at higher CD concentrations (Fig. 2). The concentration of dissolved γCD was also determined in this same complexation media that had been saturated with dexamethasone. The excipients, EDTA, BAC and HPMC, resulted in about 25% reduction in the γCD solubility at γCD concentrations above 5%. Furthermore, when the solubility profile of dexamethasone displayed decreased solubility the solubility profile of γCD displayed increased solubility. Since excess dexamethasone was added to all γCD solutions one would expect that excess dexamethasone should precipitate all γCD until its concentration corresponded to solution that had been saturated with the dexamethasone/γCD complex. This appears to be the case at γCD concentrations equal or less than 5% but not at higher concentrations. These observations indicate that the complex formation at elevated γCD concentrations does not follow the simple stoichiometry that is generally observed in ideal solutions.

Frequently pharmaceutical formulations contain combination of two or more drugs that possess different physicochemical properties, including different affinities for the formulation excipients such as CDs. Fig. 3 shows the effect of competing drugs on the phase-solubility profiles and Table 4 shows the CE calculated from the initial linear portion of the phase-solubility profiles. Hydrocortisone is a steroid with similar structure and physicochemical properties as dexamethasone (Table 1). The affinity of the two drugs to both γCD and HPγCD is of same order of magnitude, as observed by the CE values in Table 4, although on the average hydrocortisone has greater affinity for the CDs than dexamethasone. Clearly the CE is reduced when both drugs are present in the same complexation media resulting in decreased dexamethasone and hydrocortisone solubilization (Fig. 3 and Table 4). At pH 7.4 indomethacin is much less lipophilic and about 10 times more water-soluble than dex-

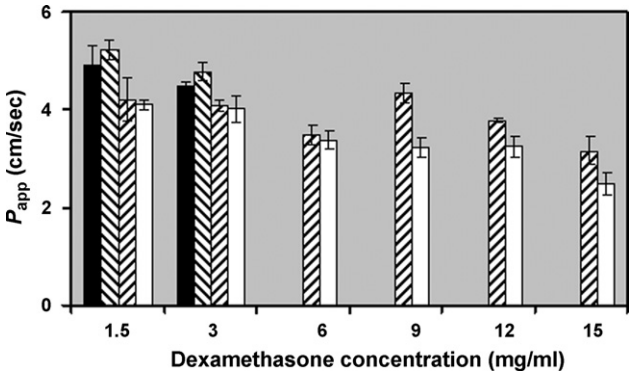


Fig. 5. Apparent permeation coefficients of dexamethasone (mean ± SD) in eye drop formulations containing different dexamethasone concentrations through semi-permeable membrane MWCO 3500; (■) γCD; (▧) γCD/HPγCD 80/20; (▨) γCD/HPγCD 20/80; (□) HPγCD.

amethasone (Table 1) and indomethacin has much lower affinity for the CDs. Indomethacin (pK_a 4.5) is in its ionized form at physiologic pH (pH 7.4) and able to form ion pair with benzalkonium in the aqueous complexation media. In addition, indomethacin exists in several different polymorphic forms that possess different intrinsic solubilities in water (Kaneniwa et al., 1985; Iohara et al., 2008). Addition of indomethacin to the complexation media has in most cases insignificant effect on the CE of dexamethasone and its solubilization, but as expected dexamethasone results in 10–20-fold decrease in the CE of indomethacin (Table 4). Amphotericin B is a lipophilic compound but its aqueous solubility is only about 1 μg/ml (Table 1). Amphotericin B has very little affinity for the CDs with CE between 0.04 and 0.07. Consequently, CD solubilization of amphotericin B is significantly reduced by the presence of dexamethasone in the aqueous complexation media resulting in up to 100-fold reduction in the CE (Fig. 3 and Table 4). Interestingly, presence of amphotericin B results in 5–76% increase in the CE of dexamethasone. It is possible that this increase in CE is due to formation of amphotericin B/dexamethasone/CD ternary complexes.

The permeability profiles in Fig. 4 show how the permeability coefficient is affected by the increasing CD concentration as well as that of competing drugs. The permeability coefficient is obtained by dividing the total concentration of dissolved drug (i.e. both free drug and drug in CD complexes and complex aggregates) into the flux (see Eq. (2)) and thus a decrease in the flux can either be due to actual decrease in the permeability coefficient or due to formation of water-soluble drug/CD aggregates that are unable to permeate the semi-permeable cellophane membrane. The general observation is that the drug/γCD complexes permeate the membrane more rapidly (i.e. have larger permeability coefficients) than the drug/HPγCD complexes and that the permeability coefficients of the individual drugs in a mixture are independent of each other. Also, the permeability coefficient of the drugs decreases with increasing CD concentration. However, amphotericin B appears to increase the permeability of dexamethasone and vice versa from complexation media containing γCD (Fig. 4A and D). At low CD concentration (<10%, w/v), the permeability coefficients of indomethacin are insignificantly higher when dexamethasone is present compared to those from pure indomethacin donor phases.

According to Table 3 the highest CE was obtained in the complete eye drop formulation that contained in addition to CD and NaCl 0.1% EDTA, 0.02% BAC and 0.1% HPMC (all %, w/v). The permeation coefficients of dexamethasone from eye drop solutions containing different types and amounts of CD are shown in Fig. 5. All the eye drop solutions tested were saturated with dexamethasone. The concentration of dissolved dexamethasone in γCD and γCD/HPγCD (80/20) containing media was determined to be between 1.5 and 3 mg/ml while it was determined to be 1.5 and 15 mg/ml in media containing either HPγCD or γCD/HPγCD (20/80). As previously observed (Fig. 4) the value of P_{app} decreased with increasing concentration of dissolved dexamethasone, possibly due to formation of water-soluble aggregates. In all cases, the mixtures of γCD/HPγCD resulted in higher P_{app} values than the pure CDs, especially when the γCD/HPγCD ratio was 80/20, which gave the highest P_{app} value (5.23 ± 0.21 cm/sec; mean ± SD). This indicates that this mixture is the optimum CD vehicle for a dexamethasone eye drop formulation.

4. Conclusions

Common pharmaceutical excipients like various salts, preservatives and water-soluble polymers can have significant effect on the solubilizing effects of CDs and the drug availability from aqueous drug formulations. The CD solubilization is also affected in combination formulations containing more than one drug. Thus, CD formulation studies should always be performed in media that closely resembles the final drug formulation.

Acknowledgement

This work was financially supported by Eimskip grant 2007, Iceland

References

Brewster, M.E., Loftsson, T., 2007. Cyclodextrins as pharmaceutical solubilizers. Adv. Drug Deliv. Rev. 59, 645–666.
Chowdary, K.P.R., Srinivas, S.V., 2006. Influence of hydrophilic polymers on celecoxib complexation with hydroxypropyl β-cyclodextrin. AAPS PharmSciTech 7, E1–E6, www.aapspharmscitech.org.
Higuchi, T., Connors, K.A., 1965. Phase-solubility techniques. Adv. Anal. Chem. Instrum. 4, 117–212.
Iohara, D., Hirayama, F., Ishiguro, T., Arima, H., Uekama, K., 2008. Preparation of amorphous indomethacin from aqueous 2,6-di-O-methyl-β-cyclodextrin solution. Int. J. Pharm. 354, 70–76.
Jansook, P., Kurkov, S.V., Loftsson, T., in press. Cyclodextrin as solubilizers: formation of complex aggregates. J. Pharm. Sci.
Jansook, P., Loftsson, T., 2008. γCD/HPγCD: synergistic solubilization. Int. J. Pharm. 363, 217–219.
Jarho, P., Pate, D.W., Brenneisen, R., Järvinen, T., 1998. Hydroxypropyl-β-cyclodextrin and its combination with hydroxypropyl methylcellulose increases aqueous solubility of Δ^9-tetrahydrocannabinol. Life Sci. 63, PL381–PL384.
Kaneniwa, N., Otsuka, M., Hayashi, T., 1985. Physicochenical charaterization of indomethacin polymorphs and the transformation kinetics in ethanol. Chem. Pharm. Bull. 33, 3447–3455.
Kristinsson, J.K., Friðriksdóttir, H., Thórisdóttir, S., Sigurðardóttir, A.M., Stefánsson, E., Loftsson, T., 1996. Dexamethasone-cyclodextrin-polymer co-complexes in aqueous eye drops. Invest. Ophthalmol. Vis. Sci. 37, 1199–1203.
Lavasanifar, A., Samuel, J., Kwon, G.S., 2002. The effect of fatty acid substitution on the in vitro release of amphotericin B from micelles composed of poly(ethylene oxide)-*block*-poly(N-hexyl stearate-L-aspartamide). J. Control. Rel. 79, 165–172.
Loftsson, T., 1998. Increasing the cyclodextrin complexation of drugs and drug bioavailability through addition of water-soluble polymers. Pharmazie 53, 733–740.
Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.
Loftsson, T., Duchêne, D., 2007. Cyclodextrins and their pharmaceutical applications. Int. J. Pharm. 329, 1–11.
Loftsson, T., Hreinsdóttir, D., 2006. Determination of aqueous solubility by heating and equilibration: a technical note. AAPS PharmSciTech 7, www.aapspharmscitech.org.
Loftsson, T., Hreinsdóttir, D., Másson, M., 2005. Evaluation of cyclodextrin solubilization of drugs. Int. J. Pharm. 302, 18–28.
Loftsson, T., Másson, M., Brewster, M.E., 2004. Self-association of cyclodextrins and cyclodextrin complexes. J. Pharm. Sci. 93, 1091–1099.
Loftsson, T., Másson, M., Sigurdsson, H.H., 2002. Cyclodextrins and drug permeability through semi-permeable cellophane membranes. Int. J. Pharm. 232, 35–43.
Loftsson, T., Másson, M., Sigurjónsdóttir, J.F., 1999. Methods to enhance the complexation efficiency of cyclodextrins. S.T.P. Pharma Sci. 9, 237–242.

Loftsson, T., Vogensen, S.B., Brewster, M.E., Konráðsdóttir, F., 2007. Effects of cyclodextrins on drug delivery through biological membranes. J. Pharm. Sci. 96, 2532–2546.
Moffat, A.C., Osselton, M.D., Widdop, B. (Eds.), 2004. Clarke's Analysis of drugs and poisons. Pharmaceutical Press, London.
Nokhodchi, A., Javadzadeh, Y., Siahi-Shadbad, M.R., Barzegar-Jalali, M., 2005. The effect of type and concentration of vehicles on the dissolution rate of a poorly soluble drug (indomethacin) from liquisolid compacts. J. Pharm. Pharmaceut. Sci. 8, 18–25.
Mura, P., Faucci, M.T., Bettinetti, G.P., 2001. The influence of polyvinylpyrrolidone on naproxen complexation with hydroxypropyl-β-cyclodextrin. Eur. J. Pharm. Sci. 13, 187–194.