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# CDs as solubilizers: Effects of excipients and competing drugs

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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

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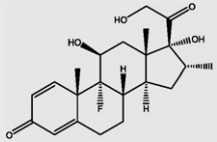
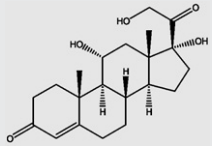
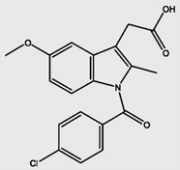
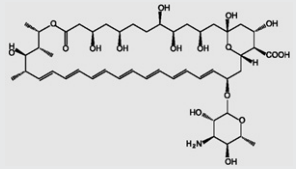
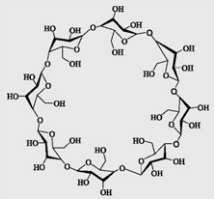
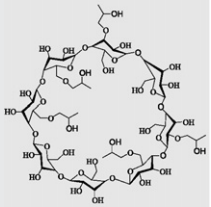
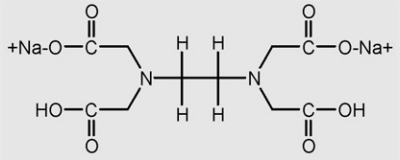
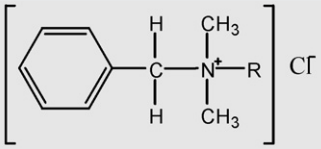
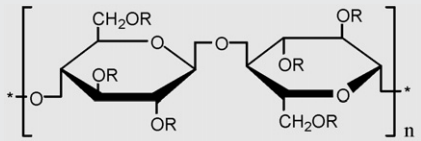
**ble 1**
ysicochemical properties of the excipients and sample compounds (Lavasanifar et al., 2002; Moffat et al., 2004; Nokhodchi et al., 2005; Brewster and Loftsson, 2007).

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

| ysicochemical properties | (B) | | |
|---|---|---|---|
| | Edetate disodium | Benzalkonium chloride | Hydroxypropylmethylcellulose |
| emical 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 $CH_3CH(OH)CH_2$ |
| olecular weight | 336.2 | 360 (average) | 10,000–1,500,000 (approximately) |
| elting point (°C)[b] | 252 (dec.) | ≈40 | 190–200 (browns) |
| a | 2.0; 2.7; 6.2; 10.3 | – | – |
| tanol/water partition coefficient | – | 9.98 for $C_{12}$; 32.9 for $C_{14}$; 82.5 for $C_{16}$ [c] | – |
| (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.

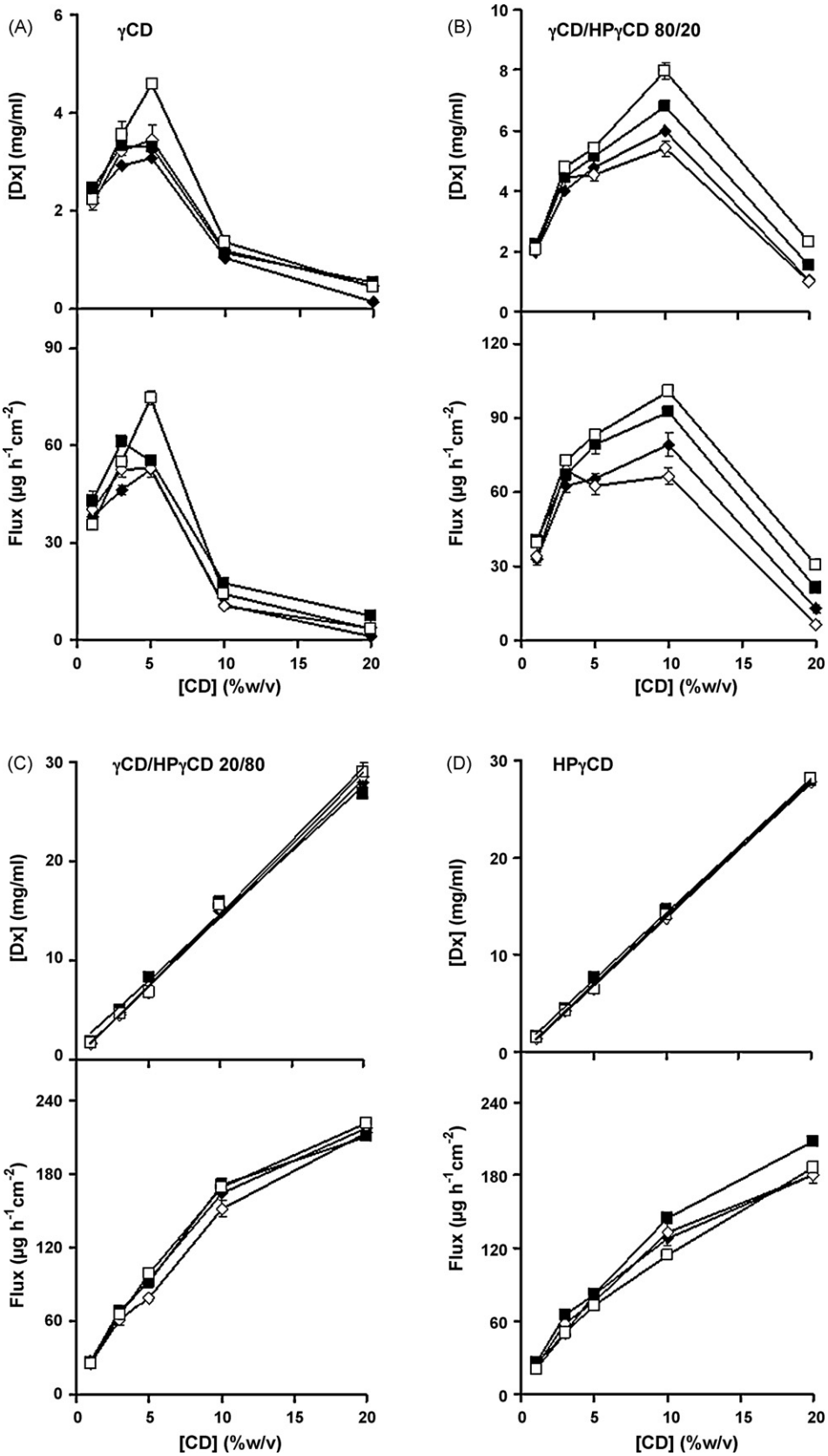



**Fig. 1.** The effect of additives in aqueous CD solution on the solubility and the flux of dexamethasone through semi-permeable cellophane membrane MWCO 3500; (A) γCD; (B) γCD/HPγCD (ratio 80:20); (C) γCD/HPγCD (ratio 20:80); (D) HPγCD; (♦) EDTA (0.1%, w/v); (◊) BAC (0.02%, w/v); (■) HPMC (0.1%, w/v);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

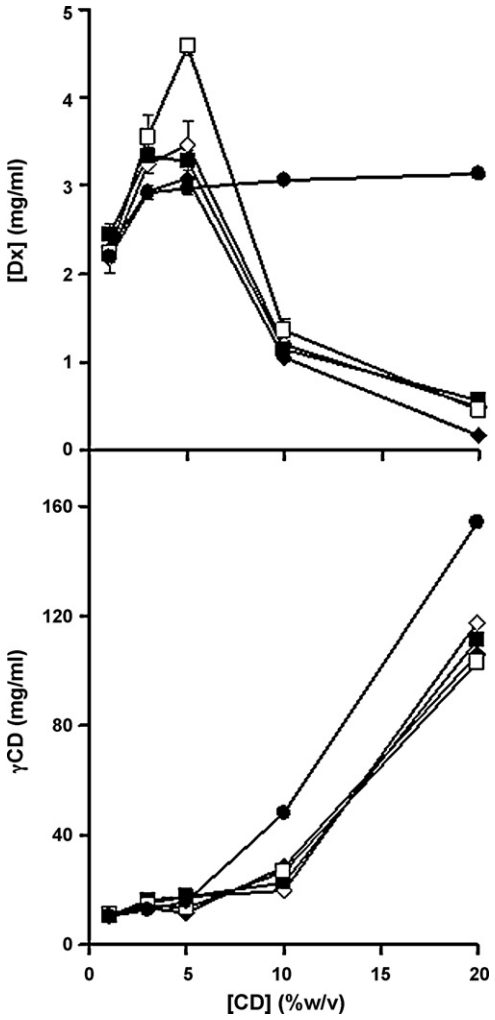



**Fig. 2.** The phase solubility diagrams of dexamethasone and the determined γCD content in aqueous γCD solution containing no additives and with additives; (●) no additive; (♦) EDTA (0.1% w/v); (◊) BAC (0.02%, w/v); (■) HPMC (0.1%, w/v); (□) all excipients (i.e. in the aqueous eye drop formulation). 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 | | | |
|---|---|---|---|---|---|---|---|---|
| | CE | Ratio | CE | Ratio | (80/20)[b] | | (20/80) | |
| | | | | | 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).

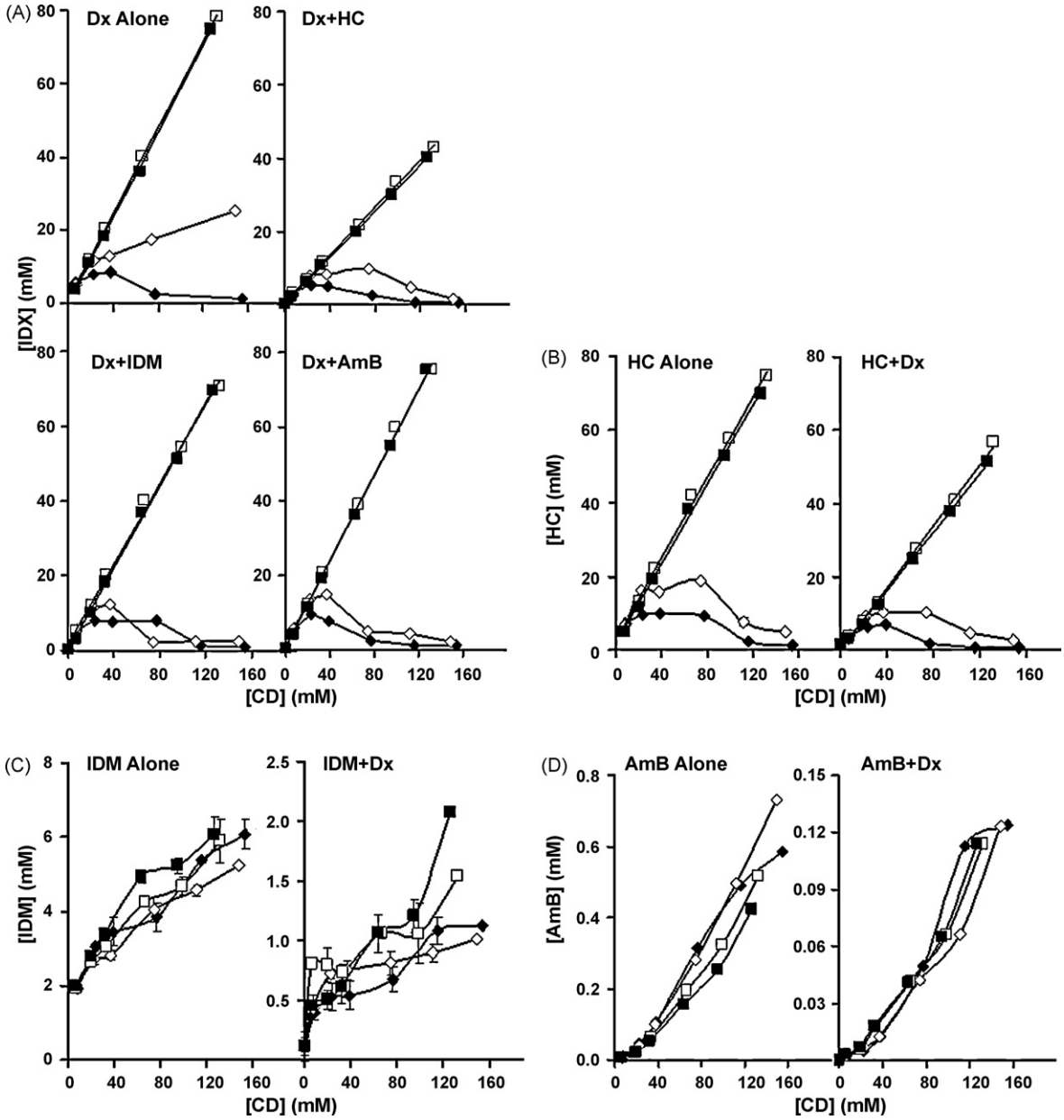



**Fig. 3.** The effect of a second drug in aqueous CD solution on phase solubility profiles of dexamethasone and its second drug in pure and different ratios of γCD/HPγCD mixtures in the aqueous eye drop formulation: (A) dexamethasone (DX); (B) hydrocortisone (HC); (C) indomethacin (IDM); (D) amphotericin 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 sol-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,

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