# Effect of 2-Hydroxypropyl-β-cyclodextrin on the Ocular Absorption of Dexamethasone and Dexamethasone Acetate

**Arunya Usayapant,[1] Adel H. Karara,[1,2] and Milind M. Narurkar[1,3]**

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Complexation of dexamethasone (DX) and dexamethasone acetate (DXA) with 2-hydroxypropyl-β-cyclodextrin (HPCD) was investigated with an ultimate goal of formulating a topical ophthalmic solution of DXA. Aqueous solubility of DX and DXA was markedly increased due to formation of soluble inclusion complexes with HPCD. Based on characterization of complex formation by phase solubility and UV-spectroscopy methods, a stoichiometry of 1:1 and 1:1, 1:2 was assumed for DX-HPCD and DXA-HPCD complexes, respectively. The stability constants for complex formation estimated by phase solubility and UV-spectroscopy methods, respectively, were as follows: for DX-HPCD complex, $K_{1:1}$ = 2193 and 2221 $M^{-1}$; and for DXA-HPCD complex, $K_{1:1}$ = 2240 and 2445 $M^{-1}$ and $K_{1:2}$ = 3 and 17 $M^{-1}$. $K_{1:1}$ of 2266 $M^{-1}$ and $K_{1:2}$ of 20 $M^{-1}$ were also estimated for the DXA-HPCD complex by kinetics. The kinetics of DXA degradation in pH 7 phosphate buffer at 25°C followed pseudo first order. The addition of HPCD decreased the rate but the order of reaction remained unchanged. Free DXA degraded at a faster rate than complexed DXA. Ocular bioavailability in conjunctiva, cornea, iris, and aqueous humor postadministration of a 25-μl dose of formulations containing an equivalent of 0.1% (w/v) DX followed a rank-order of DXA-HPCD solution > DXA suspension > DX-HPCD solution > DX suspension.

**KEY WORDS:** dexamethasone; dexamethasone acetate; complexation; 2-hydroxypropyl-β-cyclodextrin; solubility; stability; ocular bioavailability.

## INTRODUCTION

Dexamethasone (DX) is used topically to treat inflammatory conditions of the conjunctiva, cornea, and anterior segment of the eye (1). It is available for ophthalmic use as a suspension and ointment and as an ointment and aqueous solution of DX sodium phosphate. However, the polar phosphate is of little use in treating inflammation of the anterior eye segments such as uveitis (2). Present DX therapy dictates frequent instillation or application in the conjunctival sac(s), which besides leading to poor patient compliance, may result in the administration of a large dose, which, in turn, may induce glaucoma, cataract formation, and damage to the optic nerve (1).

[1] Division of Pharmaceutics and Medicinal Chemistry, School of Pharmacy, Northeast Louisiana University, Monroe, Louisiana 71209.

[2] Present address: Bioanalytics-Drug Metabolism, Sandoz Research Institute, East Hanover, New Jersey 07936.

[3] To whom correspondence should be addressed.

Dexamethasone acetate (DXA), currently unavailable for topical ophthalmic use, has been shown to readily permeate the cornea and hydrolyze to DX during and after absorption (2,3). The complexation of DX, DXA, and other steroids with either α-, β-, or Γ-cyclodextrin has been reported (4). However, hemolytic activity, nephrotoxicity, and a relatively low aqueous solubility have limited the widespread use of these cyclodextrins, particularly for ocular and parenteral administration (5). 2-Hydroxypropyl-β-cyclodextrin (HPCD) is an amorphous cyclodextrin derivative which retains the ability to form inclusion complexes but, unlike β-cyclodextrin and its methyl derivatives, is devoid of any significant toxicity (6–8). The characteristics of DX-HPCD and DXA-HPCD complex formation and aqueous stability of DXA in the presence of HPCD were investigated in order to determine the effect of HPCD on the ocular absorption of DX and DXA.

## THEORETICAL

### Characterization of Complex Formation

Complex formation between a substrate and a ligand involving 1:1 and 1:2 complex is described by the following equilibria:

$$S + L \overset{K_{1:1}}{\rightleftharpoons} SL$$

$$SL + L \overset{K_{1:2}}{\rightleftharpoons} SL_2$$

where $S$ represents a substrate (DX or DXA) and $L$ represents the ligand (HPCD). $K_{1:1}$ and $K_{1:2}$ can be determined by several methods such as phase solubility (9), UV spectroscopy (10,11), and kinetics (12). For 1:1 complex, the total substrate concentration in solution ($S_t$) as a function of the total ligand concentration ($L_t$) can be written as

$$[S_t] = [S_o] + \frac{K_{1:1}[S_o][L_t]}{1 + K_{1:1}[S_o]} \quad (1)$$

where $S_o$ is the inherent aqueous solubility of the substrate.

For 1:2 complex, assuming $[L_t] \approx [L]$, the equation is

$$\frac{[S_t] - [S_o]}{[L_t]} = K_{1:1}[S_o] + K_{1:1}K_{1:2}[S_o][L_t] \quad (2)$$

where $[L]$ is the free ligand concentration.

$K_{1:1}$, for a 1:1 complex, can also be estimated using UV spectroscopy by the Benesi–Hildebrand equation (10):

$$\frac{b}{dA} = \frac{1}{[S_t]K_{1:1}d\epsilon_1[L_t]} + \frac{1}{[S_t]d\epsilon_1} \quad (3)$$

where $dA$ represents the decrease in absorbance of $S_t$ as a function of ligand concentration ($L_t$). $b$ is the width of the quartz cell.

For a 1:2 complex, assuming $[L_t] \approx [L]$, the equation is

$$\frac{dA}{b[S_t]} = \frac{d\epsilon_1K_{1:1}[L_t] + d\epsilon_2K_{1:1}K_{1:2}[L_t]^2}{1 + K_{1:1}[L_t] + K_{1:1}K_{1:2}[L_t]^2} \quad (4)$$



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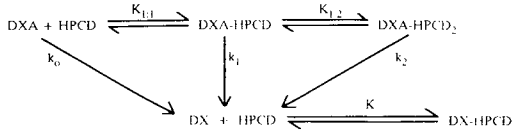
$K_{1:1}$ and $K_{1:2}$ can be estimated from Eq. (4) by nonlinear regression (PCNONLIN) (11).

### Degradation Kinetics of DXA in the Presence of HPCD

The degradation kinetics of DXA in the presence of HPCD can be described schematically as shown below:




Scheme I

where $k_o$ is the observed rate constant for degradation of uncomplexed DXA, $K$ is the stability constant for DX-HPCD complex formation, and $k_1$ and $k_2$ are the rate constants for DXA degradation from 1:1 and 1:2 complexes, respectively. The general rate equation for the degradation of the total drug, $[DXA]_T$, can be described as

$$\frac{-d[DXA]_T}{dt} = k_o[DXA] + k_1[DXA\text{-}HPCD] + k_2[DXA\text{-}HPCD_2] \quad (5)$$

where

$$[DXA]_T = [DXA] + [DXA\text{-}HPCD] + [DXA\text{-}HPCD_2] \quad (6)$$

Equation (5) can be rearranged to

$$\frac{-d[DXA]_T}{dt} = \frac{k_o + k_1K_{1:1}[HPCD] + k_2K_{1:1}K_{1:2}[HPCD]^2}{1 + K_{1:1}[HPCD] + K_{1:1}K_{1:2}[HPCD]^2}[DXA]_T = k_{obs}[DXA]_T \quad (7)$$

where

$$k_{obs} = \frac{k_o + k_1K_{1:1}[HPCD] + k_2K_{1:1}K_{1:2}[HPCD]^2}{1 + K_{1:1}[HPCD] + K_{1:1}K_{1:2}[HPCD]^2} \quad (8)$$

$k_{obs}$ is the observed rate constant for overall DXA degradation. $k_1$, $k_2$, $K_{1:1}$ and $K_{1:2}$ can be estimated by nonlinear regression (PCNONLIN) of Eq. (8).

## MATERIALS AND METHODS

### Materials

HPCD (average MW = 1538) was purchased from Pharmatec Inc., Alachua, FL. DX and DXA were purchased from Sigma Chemical Co., St. Louis, MO. 1,2,4,6,7-$^3$H-DX, tissue solubilizer, and scintillation fluid were purchased from Amersham Corporation, Arlington Heights, IL. $^3$H-DXA was synthesized from $^3$H-DX. HPLC solvents were of HPLC grade. All other chemicals were of reagent grade. Distilled deionized water was used to prepare mobile phases and buffer solutions. Albino (NZW) rabbits weighing 2.0–2.5 kg had free access to food and water and were kept in restraining boxes in an upright position during experimentation.

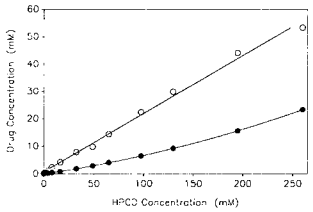



Fig. 1. Phase solubility diagram of DX (○) in water and of DXA (●) in 0.1 *M* citrate buffer, pH 6.0, at 25°C. Each data point represents a mean ($n$ = 3), with SD smaller than the symbol size.

### Synthesis of $^3$H-DXA

Acetylation was effected by adding acetic anhydride to a solution of $^3$H-DX in pyridine and triethylamine, with the reaction run at low temperature. $^3$H-DXA was purified by TLC. Silica gel GF, 250 μm (Analtech, Newark, DE), was used as a stationary phase, with chloroform:acetone (9:1) as a developing solvent. The calculated $R_f$ value was 0.1 and 0.38 for DX and DXA, respectively.

### HPLC Analysis

HPLC was used to determine quantitatively DX and DXA in *in vitro* studies. The HPLC setup included a Varian 5000 pump, Valco injector valve, Varian UV-50 variable-wavelength detector, Water 740 data integrator, and Rainin Microsorb Cyano column (5 μm). The mobile phase was 65% (v/v) methanol in water at a flow rate of 1 ml/min, with the effluent being monitored at 246 nm. Retention times of DX and DXA were 5 and 6 min, respectively.

### Liquid Scintillation Counting (LSC)

Ocular tissues were digested by incubation with 1 ml of tissue solubilizer at 45°C for 24 hr. The samples were then vortexed and allowed to equilibrate at room temperature. Fifteen milliliters of scintillation fluid was added to each

Table I. Stability Constants for Complex Formation by Phase Solubility, UV Spectroscopy, and Kinetic Methods at 25°C

| Complex system | Constant for complex formation[a] ($M^{-1}$) | | |
|---|---|---|---|
| | Phase solubility | UV spectroscopy | Kinetic[b] |
| DX-HPCD (in water) | | | |
| $K_{1:1}$ | 2193 (22) | 2221 (118) | — |
| DXA-HPCD (in 0.1 *M* citrate buffer, pH 6.0) | | | |
| $K_{1:1}$ | 2240 (111) | 2445 (115) | 2266 (87) |
| $K_{1:2}$ | 3 (0) | 17 (8) | 20 (3) |

[a] Reported as the mean ($n$ = 3), with the standard deviation in parentheses.

[b] Investigated in 0.1 *M* phosphate buffer at pH 7.0.

sample. All samples were vortexed and stored in the dark for at least 24 hr to minimize photoluminescence. Following storage, the samples were counted for radioactivity using Beckman LS 3801 (Beckman Instrument Inc., Fullerton, CA). Tritium standard ($10^5$ dpm) was used to calibrate a quench curve and the counting efficiency.

Blood samples (500 μl) obtained before and after instillation of the dose were digested by incubation with 500 μl of 60% perchloric acid and 500 μl of 30% hydrogen peroxide, at 45°C for 24 hr. One hundred microliters of freshly prepared ascorbic acid (15%) was added to the digested blood to reduce chemical luminescence (13).

### Phase Solubility Studies

These studies were performed according to the method reported by Higuchi and Connors (9) at 25°C, with various concentrations of HPCD (0–260 m*M*). Deionized water was used for DX and 0.1 *M* citrate buffer (pH 6.0) for DXA to minimize the degradation of DXA during the experiment. The citrate buffer species were found not to complex with DXA and, therefore, did not increase DXA solubility per se. Three determinations were made at each HPCD concentration.

### Characterization of Complex Formation by UV Spectroscopy

A decrease in UV absorbance of an aqueous solution of DX (0.27 m*M*) in the presence of HPCD (0.016–0.13 m*M*) was recorded at a $\lambda_{max}$ of 243 nm. Similar experiment was performed with DXA (0.023 m*M*) in citrate buffer (pH 6.0), containing various concentrations of HPCD (0.033–4.9 m*M*). The experiments were repeated in triplicate for each drug.

### Degradation Kinetics of DXA in Aqueous Solution

Hydrolysis of DXA was studied at 25°C in phosphate buffer (pH 6.0 and 7.0), acetate buffer (pH 6.0), and citrate buffer (pH 5.0 and 6.0). Pseudo first-order rate constants for DXA hydrolysis were calculated by linear regression of the logarithm of peak area versus time plots. The experiments were performed in triplicate and the mean value of the rate constant was determined. The effect of HPCD (0–260 m*M*) on DXA degradation in pH 7 phosphate buffer at 25°C was also examined.

### Preparation of Formulations for Topical Instillation

Tritium labeling was accomplished by adding an adequate amount of the tritiated drug ($^3$H-DX or $^3$H-DXA) to an alcoholic solution of nontritiated drug. The solution was evaporated to dryness under nitrogen at room temperature. The resulting tritium-labeled drug was found to have a radioactivity of 0.14 mCi/mg. Four representative formulations, containing an equivalent of 0.1% (w/v) DX, were prepared using the radiolabeled drug(s).

Normal saline and 0.1 *M* citrate buffer (pH 6) were used as the vehicle for a suspension of $^3$H-DX and $^3$H-DXA, respectively. Fine particles were obtained by grinding with a glass rod and sonicating for 45 min, a period which was found sufficient to achieve saturation solubility. Particle size distribution was determined by microscopic method (14). At least 200 particles were measured and no evidence of agglomeration was observed.

A solution of complex was prepared by equilibrating at 25°C an excess amount of $^3$H-DX or $^3$H-DXA in the appropriate vehicle containing a sufficient amount of HPCD (5%, w/v, for DX and 8%, w/v, for DXA). After 3 days, the so-

Table II. Observed Rate Constants for DXA Degradation in Various Buffer Systems at 25°C, with and Without HPCD

| Buffer system | HPCD (m*M*) | $\times 10^3$ (day$^{-1}$)[a] | |
|---|---|---|---|
| | | $k_o$ | $k_{obs}$ |
| 0.1 *M* acetate buffer, pH 6.0 | — | 12.8 (1.0) | |
| 0.1 *M* citrate buffer, pH 5.0 | — | 1.9 (0) | |
| 0.1 *M* citrate buffer, pH 6.0 | — | 2.0 (0.1) | |
| 0.1 *M* phosphate buffer, pH 6.0 | — | 208.1 (26.9) | |
| 0.1 *M* phosphate buffer, pH 7.0 | — | 14.6 (0.4) | |
| | 0.81 | | 8.5 (0.2) |
| | 16.0 | | 4.8 (0.2) |
| | 32.0 | | 4.3 (0.1) |
| | 98.0 | | 3.5 (0.1) |
| | 260.0 | | 3.0 (0.4) |

[a] Reported as the mean ($n$ = 3), with the standard deviation in parentheses.

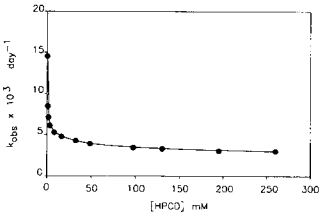



Fig. 2. Plot showing nonlinear dependency of $k_{obs}$ for DXA degradation on HPCD concentration in 0.1 *M* phosphate buffer, pH 7.0, at 25°C. Each data point represents a mean ($n$ = 3), with the SD smaller than the symbol size.

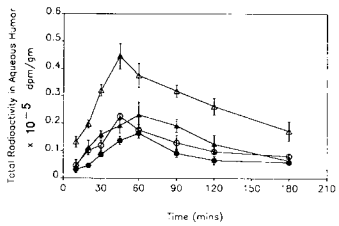



Fig. 3. Mean radioactivity ($n$ = 4) per gram of aqueous humor postinstillation of 25-μl dose of DX suspension (●), DX-HPCD solution (○), DXA suspension (▲), and DXA-HPCD solution (△).

lution of drug-HPCD complex was filtered and analyzed for drug concentration by UV spectroscopy at 243 nm. The filtrates were diluted with the appropriate vehicle to obtain the final drug concentration equivalent to 0.1% (w/v) DX. By knowing the dilution factor, the final concentration (%, w/v) of HPCD was calculated to be 1.5% (9.8 m*M*) for $^3$H-DX-HPCD complex and 7.0% (46 m*M*) for $^3$H-DXA-HPCD complex.

### Determination of Ocular Bioavailability

Twenty-five microliters of a test formulation was topically instilled into rabbit eyes. Both eyes of the rabbit received the same formulation, with the doses administered 5 min apart, to aid in sample procurement. At predetermined time intervals postinstillation, rabbits were killed with an injection of sodium pentobarbital into the marginal ear vein. The eye tissues and aqueous humor were obtained according to a published method (15). Radioactivity is reported as disintegrations per minute per gram of wet tissue or fluid. The mean radioactivity ($n$ = 4) in each tissue and aqueous humor was plotted as a function of time. The area under the radioactivity–time curve up to 3 hr ($AUC_{0-3}$) was calculated by trapezoidal rule. The differences in $AUC_{0-3}$ among formulations were subjected to a statistical test using ANOVA and SNK (SAS PC-computer package).

Blood samples (500 μl) were obtained from a precannulated ear artery at several times postinstillation, for each formulation. A blood sample was also obtained before instillation of the dose and was used as a blank to correct for the background count.

## RESULTS AND DISCUSSION

### Characterization of Complex Formation

The solubility of both drugs increased as a function of HPCD concentration, with the generation of an $A_L$-type and an $A_P$-type diagram for DX and DXA, respectively, as shown in Fig. 1. At 260 m*M* HPCD, the solubility enhancement for DX and DXA was 377- and 1016-fold, respectively, based on a $S_o$ of 0.16 m*M* for DX and 0.023 m*M* for DXA. In UV spectroscopy, a precise isosbestic point in DX-HPCD system and multiple spectral intersections in the DXA-HPCD system were observed. Based on the aforementioned results, a stoichiometry of 1:1 for DX-HPCD and 1:1, 1:2 for DXA-HPCD complex was assumed.

Table I presents the values of $K_{1:1}$ and $K_{1:2}$ determined by different methods. The values reported for a particular substrate by each method were in close agreement except for the $K_{1:2}$ of DXA-HPCD complex, where the phase solubility method gave a lower estimate. Differences in the values of the stability constant(s) determined by different methods have been observed by Connors and Mollica (12). The relatively small magnitude of $K_{1:2}$ indicates that the formation of 1:2 complex is less favored.

### Effect of HPCD on the Aqueous Stability of DXA

Hydrolysis of DXA was found to follow pseudo first-

Table III. Extent of Absorption and Relative Bioavailability Following 25-μl Dose of Four Formulations[a] in Ocular Tissues and Aqueous Humor

| Tissue or fluid | $(AUC)_{0-3}$[b] ($\times 10^{-5}$ dpm/gm * min) | | | | Relative bioavailability | | |
|---|---|---|---|---|---|---|---|
| | DX | DX-HPCD | DXA | DXA-HPCD | DX-HPCD/ DX | DXA-HPCD/ DXA | DXA-HPCD/ DX-HPCD |
| Conj. | 316.8 (38.9) | 662.9 (52.4) | 894.7 (137.5) | 1499.5 (188.4) | 2.1 | 1.7 | 2.3 |
| Cornea | 309.2 (32.6) | 581.8 (51.8) | 725.3 (134.4) | 1332.2 (137.3) | 1.9 | 1.8 | 2.3 |
| Iris | 180.4 (14.5) | 267.4 (22.1) | 385.6 (55.2) | 702.5 (60.1) | 1.5 | 1.8 | 2.6 |
| Aq. humor | 15.0 (2.0) | 20.9 (2.3) | 27.3 (5.2) | 53.8 (5.9) | 1.4 | 2.0 | 2.6 |

[a] Formulations contained an equivalent of 0.1% (w/v) DX.
[b] Reported as the mean ($n$ = 4), with the standard deviation in parentheses.

order kinetics in aqueous buffer solutions. The observed rate constants for DXA degradation ($k_o$) in various buffer systems are shown in Table II. At pH 6.0 and an equimolar buffer concentration, DXA was found to be most stable in citrate buffer and least stable in phosphate buffer.

The introduction of HPCD (0.81–260 m*M*) to a phosphate buffered pH 7 solution led to a decrease in the rate of DXA hydrolysis. A nonlinear relationship between $k_{obs}$ and HPCD concentration was obtained as depicted in Fig. 2. Analysis of the curve by PCNONLIN yielded the estimates of $k_1$, $k_2$, $K_{1:1}$, and $K_{1:2}$. The estimated $K_{1:1}$ and $K_{1:2}$ values by this method are included in Table I. The magnitude of $k_1$ and $k_2$ was estimated to be 5.2 and $2.5 \times 10^{-3}$ $day^{-1}$, respectively. Since $k_o$ has been shown to be $14.6 \times 10^{-3}$ $day^{-1}$, these results indicate that formation of 1:2 complex decreased DXA degradation by sixfold, while the 1:1 complex provided a threefold decrease at pH 7 and 25°C. Based on Table II, formulations of DXA for topical instillation were prepared in 0.1 *M* citrate buffer (pH 6).

### Ocular Bioavailability of Formulations

It was evident that absorption from the solution of DXA-HPCD complex was greater in all tissues and aqueous humor, relative to the other three formulations. A typical radioactivity–time curve for all formulations in aqueous humor is shown in Fig. 3. A rank-order comparison of absorption efficiency indicates DXA-HPCD solution > DXA suspension > DX-HPCD solution > DX suspension, in all tissues and aqueous humor. For a particular dosage form, the DXA formulation appears to be more bioavailable than DX. As expected, the two solution formulations exhibited a faster absorption rate, as evidenced by a shorter $t_{max}$, when compared with their corresponding suspension formulation.

Table III lists the $AUC_{0-3}$ for all formulations in each tissue and aqueous humor. Also listed in this table is the apparent bioavailability enhancement under three comparisons. The differences in $AUC_{0-3}$ for all formulations were deemed to be statistically significant ($P < 0.05$) by ANOVA.

In *in vitro* studies, DXA has been shown to permeate the excised cornea approximately eight times faster than DX (3), which may explain the increased absorption from DXA suspension as compared to the suspension of DX. Cyclodextrins and their complexes are known to be unable to penetrate cell membranes in an effective manner (16,17). The precorneal fluid volume is too low to cause any significant dissociation of the administered complex. However, lipids have been reported to form highly stable complexes with cyclodextrins (18) and may displace the drug from the complex, thereby increasing the free drug level in the tear film and at the corneal surface. Bioconversion of DXA to DX during corneal permeation would further enhance the flux of the displaced DXA from the precorneal area into the corneal membrane. Therefore, the comparatively greater absorption from DXA-HPCD solution than DX-HPCD solution can be attributed to the higher permeability of DXA and the influence of bioconversion on its flux from the precorneal area.

The average particle size in the two suspensions reported as mean volume surface diameter ($d_{vs}$), was 21.9 and 21.7 μm for DX and DXA, respectively. Preliminary studies indicated a slightly increased viscosity in the formulations containing HPCD. These factors were therefore deemed to be relatively insignificant contributors to bioavailability. The radioactivity in the blood postinstillation was also found to be insignificant compared to levels in the ocular tissues.

In conclusion, the results of this study suggest the feasibility of formulating a DXA-HPCD dosage form for topical ophthalmic use.

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