# Hydroxypropyl-β-Cyclodextrin Increases the Aqueous Solubility and Stability of Pilocarpine Prodrugs

**Pekka Jarho,[1,3] Arto Urtti,[2] and Tomi Järvinen[1]**

*Received February 10, 1995; accepted April 12, 1995*

***Purpose.*** The effects of 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) on the aqueous solubility and stability of two lipophilic bispilocarpine prodrugs were investigated at pH 7.4. ***Methods.*** The solubility of prodrugs was studied by phase-solubility method (0–72.5 mM HP-β-CD). The stability of one of the prodrugs was investigated as a function of temperature (40°C–70°C) and HP-β-CD concentration (0–72.5 mM). The apparent rate constants ($k_1$, $k_2$) for degradation of prodrug in 1:1 and 1:2 inclusion complexes and apparent stability constants ($K_{1:1}$, $K_{1:2}$) were calculated by the curve-fitting method. ***Results.*** The phase-solubility diagrams were classified as $A_p$-type and the apparent stability constants ($K_{1:1}$, $K_{1:2}$) for 1:1- and 1:2-inclusion complexes were calculated to be 143–815 $M^{-1}$ and 29–825 $M^{-1}$, respectively. The stability of prodrug increased as a function of HP-β-CD concentration over the studied temperature range. The shelf-life ($t_{90\%}$, calculated by the Arrhenius equation) of the prodrug in 72.5 mM HP-β-CD solution increased 5.1-fold and 6.1-fold at 25°C and 4°C, respectively. ***Conclusions.*** The solubility of the prodrugs was shown to increase markedly in phase-solubility studies. The degradation rate of prodrug in stability studies was shown to be slower in the 1:2-complex than in the 1:1-complex and the relative amounts of complex species were found to be dependent on CD concentration.

**KEY WORDS:** prodrug; bispilocarpic acid diester; hydroxypropyl-β-cyclodextrin; inclusion complex; solubility; stability.

## INTRODUCTION

Pilocarpine is a widely used drug for the treatment of glaucoma. However, the ocular bioavailability of pilocarpine is very low (1). The major disadvantage of pilocarpine is its poor corneal permeability, probably due to the low lipophilicity of the molecule. Thus a prodrug approach has been used to improve the ocular delivery of pilocarpine (2–4). Bispilocarpic acid diesters are lipophilic dimeric pilocarpine double prodrugs containing two pilocarpine molecules. These prodrugs release pilocarpine via enzymatic and chemical hydrolysis in the eye (5). The corneal permeability of these lipophilic pilocarpine prodrugs are several times higher than that of pilocarpine (6). Unfortunately these prodrugs cause significant eye irritation and have low aqueous solubility and stability at the physiological pH, which is most desirable for ophthalmic solution administration. The solubility and stability of prodrugs can be increased by lowering the pH of solution, but this will also decrease the ocular absorption of the prodrugs. An earlier study showed that the irritation due to one of these prodrugs could be suppressed by the use of SBE4-β-CD, a sulfobutyl ether derivatives of β-cyclodextrin (7).

It has been reported that co-administration of 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) increases the intraocular pressure lowering effect of prostaglandins (8) and carbonic anhydrase inhibitors (9) and ocular absorption of dexamethasone, dexamethasoneacetate (10,11) and diclofenac sodium (12). In the case of pilocarpine, cyclodextrins (CDs) have been reported to enhance (13) pilocarpine delivery but a recent study has challenged this observation (14). The use of CDs in ophthalmic preparations has been reviewed recently (15), where it was concluded that one suitable ophthalmic application of CDs may be the preparation of solutions of highly insoluble prodrugs.

The aim of the present study was to increase the aqueous solubility and stability of lipophilic pilocarpine prodrugs at physiological pH with HP-β-CD.

## MATERIALS AND METHODS

### Chemicals

The pilocarpine prodrugs, O,O′-dipivalyl (1,4-xylylene)- and O,O′-dicyclobutanecarbonyl (1,2-ethylene) bispilocarpic acid diester (Figure 1) were synthesized and identified according to previously described methods (16). HP-β-CD (Molecusol®; MW = 1379, degree of molar substitution 0.6) was purchased from Pharmatec Inc. (Alachua, FL). Disodium phosphate dihydrate was obtained from Merck (Darmstat, Germany). Sodium chloride and methanol (HPLC-grade) were acquired from T.J. Baker (Denventer, The Netherlands). Ethanol was purchased from OY Alko AB (Finland). Hydrochloric acid was obtained from Riedel-de Haen (Seelze, Germany) and sodium hydroxide from Eka Nobel AB (Bohus, Sweden).

### Apparatus

High performance liquid chromatography (HPLC) was performed with a system consisting of the Beckman programmable solvent module 116, a Beckman programmable UV detector (set at 215 nm), the System Gold data module (Beckman Instruments Inc. San Ramon, USA), a Marathon autosampler equipped with column thermostat (Spark Holland, Emmen, The Netherlands), and a Rheodyne 7080-080 loop injector. A deactivated Supelcosil LC8-DB (15 cm × 4,6 mm i.d., 5 μm) reversed-phase column (Supelco, Bellefonte, USA) was used for the separations. The chromatographic conditions were as follows: injection volume, 20 μl; column temperature, 40°C; flow rate, isocratic at 1.0 ml/min. The mobile phase used consisted of 29% monobasic potassium phosphate buffer (0.02 M, pH 4.5) in methanol. An Orion SA 520 pH meter (Boston, USA) equipped with a combination pH electrode, was used for pH determinations.

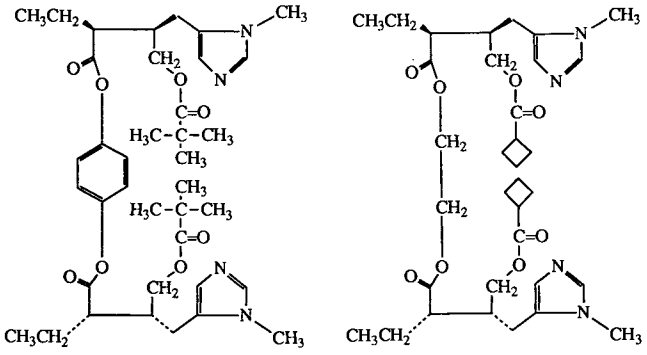
[1] Department of Pharmaceutical Chemistry, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland.

[2] Department of Pharmaceutical Technology, University of Kuopio, P.O.Box 1627, FIN-70211 Kuopio, Finland.

[3] To whom correspondence should be addressed at the Department of Pharmaceutical Chemistry, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland.



0724-8741/95/0900-1371$07.50/0 



A B

Fig. 1. Chemical structure of O,O'-dipivalyl (1,4-xylylene) bispilocarpic acid diester (Prodrug A) and O,O'-dicyclobutanecarbonyl (1,2-ethylene) bispilocarpic acid diester (Prodrug B).

### Solubility Studies

The stability constants for inclusion complex formation between the pilocarpine prodrugs (prodrugs A and B) and HP-β-CD were determined using the phase-solubility method (17). Excess amount of the prodrug was added to phosphate buffer solutions (0.16 M, ionic strength of 0.5, pH 7.4) containing various concentration (3.6–72.5 mM) of HP-β-CD. The suspensions were shaken at 25°C for 72 h and the pH of the suspension was monitored during equilibration. The pH of suspensions was adjusted to 7.4 with HCl or NaOH, if necessary. After equilibration, the suspensions were filtered through 0.45 μm membrane filters and analysed by HPLC. The solubility of prodrugs in phosphate buffer (0.16 M, pH 7.4), in the absence of HP-β-CD ($S_o$), were determined as an average of five determinations.

### Stability Studies

The stability of O,O'-dicyclobutanecarbonyl (1,2-ethylene) bispilocarpic acid diester (prodrug B), in phosphate buffer (0.16 M, ionic strength 0.5, pH 7.4) was studied as a function of temperature (40°C–70°C) and HP-β-CD concentration (0–72.5 mM). Solutions of the bispilocarpic acid diester fumarate were prepared by dissolving an appropriate amount of the prodrug B in 1.0 ml of ethanol followed by an addition of 25 ml of the pre-heated buffer-solution containing the desired cyclodextrin concentration. Ethanol was used due to poor solubility of the prodrug in the absence of HP-β-CD. The solutions were placed in a constant temperature and at the appropriate intervals, 1 ml samples were taken. The remaining prodrug was assayed according to the peak areas measured by the HPLC method described previously. The pseudo-first-order rate constants ($k_{obs}$) and half-lives ($t_{1/2}$) for the overall degradation of prodrug B were determined from the slopes of the linear semilogarithmic plots of the remaining prodrug versus time. The values for degradation rate of prodrug in inclusion complexes ($k_1$, $k_2$) and the stability constants for inclusion complexes ($K_{1:1}$, $K_{1:2}$) were calculated from degradation data (Macintosh KaleidaGraph).

## RESULTS AND DISCUSSION

### Phase-Solubility Studies

Figure 2 shows the phase-solubility diagrams of O,O'-dipivalyl (1,4-xylylene) bispilocarpic acid diester (prodrug A) and O,O'-dicyclobutanecarbonyl (1,2-ethylene) bispilocarpic acid diester (prodrug B) in phosphate buffer (0.16 M, ionic strength 0.5, pH 7.4). The phase-solubility diagrams are $A_p$-type (17) indicating formation of 1:1 and 1:2 prodrug/HP-β-CD complexes. The stability constants for the 1:1 and 1:2 complexes were calculated after constructing a plot by using equation 1 (17):

$$\frac{([S_t] - [S_0])}{[L_t]} = K_{1:1}[S_0] + K_{1:1}K_{1:2}[S_0][L_t] \qquad (1)$$

where $[S_t]$ is the total drug concentration at total cyclodextrin concentration $[L_t]$, $[S_o]$ is the solubility of prodrug in the absence of cyclodextrin and $K_{1:1}$, and $K_{1:2}$ represents the stability constants for 1:1-complex and 1:2-complex, respectively. A plot of $([S_t]-[S_o])/[L_t]$ v. $[L_t]$ results in a linear plot with an intercept of $K_{1:1}[S_o]$ and a slope of $K_{1:1}K_{1:2}[S_o]$. The stability constants for 1:1- and 1:2-complexes were calculated to be 815 $M^{-1}$ and 825 $M^{-1}$ for O,O'-dipivalyl (1,4-xylylene) bispilocarpic acid diester and 143 $M^{-1}$ and 29 $M^{-1}$ for O,O'-dicyclobutanecarbonyl (1,2-ethylene) bispilocarpic acid diester, respectively.

Complex-formation involving 1:1 and 1:2 complexes can be represented by the following equilibria:

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

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

where $S$ represents a substrate (prodrug), $L$ represents the free ligand (HP-β-CD) and $SL$ and $SL_2$ are 1:1 and 1:2 inclusion complexes formed by the substrate and the ligand, re-

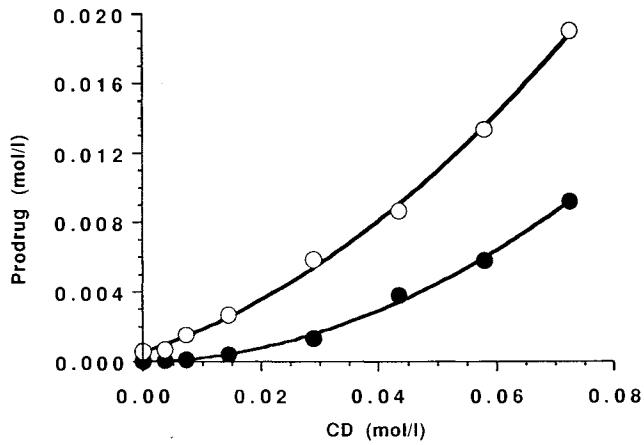



Fig. 2. Phase-solubility diagrams of O,O'-dipivalyl (1,4-xylylene) bispilocarpic acid diester (●) and O,O'-dicyclobutanecarbonyl (1,2-ethylene) bispilocarpic acid diester (○) in phosphate buffer (0.16 M, pH 7.4).

spectively. The general definitions for equilibrium constants $K_{1:1}$ and $K_{1:2}$ are given by the following two equations:

$$K_{1:1} = \frac{[SL]}{[S][L]} \quad (2)$$

$$K_{1:2} = \frac{[SL_2]}{[SL][L]} \quad (3)$$

Where $[SL]$ and $[SL_2]$ are the concentration of the complexes, $[S]$ is the concentration of the free substrate and $[L]$ is the concentration of free HP-β-CD.

In the phase-solubility method, excess drug is added into a solution containing different cyclodextrin concentration. The intrinsic solubility of the prodrug ($S_o$) is a constant over the range of HP-β-CD concentrations. Including Eq. 1, the assumption is made that $[L] \approx [L_T]$. This is reasonable if $[L_T] > [S_T]$ and the interaction constant is rather weak. Then the concentration of 1:1-complex and 1:2-complex can be calculated from equation 2 and 3 at different HP-β-CD concentrations. Figure 3 shows the calculated concentrations of 1:1 and 1:2 prodrug B/HP-β-CD complexes by using stability constants 143 $M^{-1}$ ($K_{1:1}$) and 29 $M^{-1}$ ($K_{1:2}$). The total solubility of prodrug B is calculated by using the concentration of 1:1-complexes, 1:2-complexes and intrinsic solubility. The calculated solubility is in good agreement with the experimentally determined solubility of the pilocarpine prodrug B (Figure 3).

### Stability Studies

The effects of HP-β-CD on the stability of O,O'-dicyclobutanecarbonyl (1,2-ethylene) bispilocarpic acid diester (prodrug B) was studied as a function of temperature (40°C–70°C) and HP-β-CD concentration (0–72.5 mM) at pH 7.4. The degradation of pilocarpine prodrug B followed first-order kinetics in the presence and absence of HP-β-CD. The degradation rate of prodrug B decreased by increasing the HP-β-CD concentration at temperatures studied.

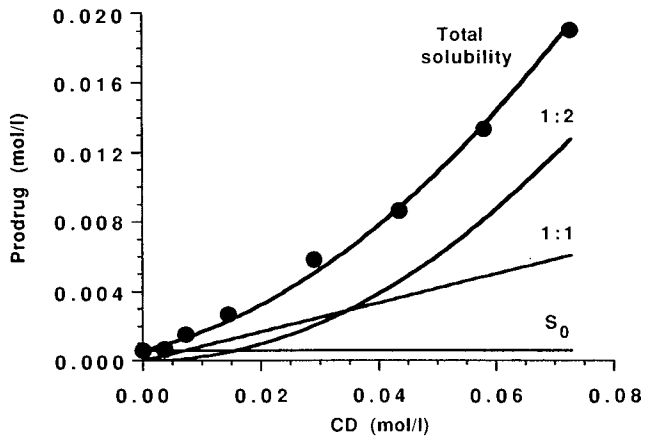
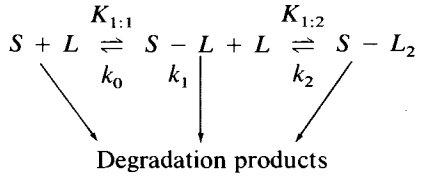



Fig. 3. Calculated concentrations (solid lines) of 1:1-complexes, 1:2-complexes and total solubility of the prodrug at different HP-β-CD concentrations in phase-solubility studies of O,O'-dicyclobutanecarbonyl (1,2-ethylene) bispilocarpic acid diester at pH 7.4. Experimental data (●) and intrinsic solubility ($S_o$) included for comparison.

The degradation rate of a drug, which follows first order kinetics, can be expressed as in equation 4:

$$\frac{-d[\text{Drug}]_T}{dt} = k_{obs}[\text{Drug}]_T \quad (4)$$

where $k_{obs}$ is the observed rate constant for the degradation of drug. Complexation of the drug with cyclodextrin can increase drug stability by lowering the degradation rate of the molecules within the inclusion complex. The degradation kinetics of a drug ($S$) forming both 1:1 and 1:2 complexes with cyclodextrin ($L$) can be described as shown in scheme I:




Scheme I

where $k_o$ is the rate constant for degradation of uncomplexed drug. The rate constants ($k_1$ and $k_2$) reflect degradation of the drug in the inclusion complexes that incorporate one and two cyclodextrin molecules, respectively. $K_{1:1}$ and $K_{1:2}$ are the stability constants for 1:1- and 1:2-complexes. The total degradation of drug ($S$) can be described by Eq. 5:

$$\frac{-d[S]_T}{dt} = k_0[S] + k_1[SL] + k_2[SL_2] \quad (5)$$

where

$$[S]_T = [S] + [SL] + [SL_2] \quad (6)$$

with Eqs. 2, 3, and 6, Eq. 5 can be rearranged to Eq. 7, where the degradation rate of drug has been described as a function of total drug concentration (10):

$$\frac{-d[S]_T}{dt} = \frac{k_0 + k_1K_{1:1}[L] + k_2K_{1:1}K_{1:2}[L]^2}{1 + K_{1:1}[L] + K_{1:1}K_{1:2}[L]^2}[S]_T \quad (7)$$

Since Eq. 7 has a same form as Eq. 4, the observed overall degradation rate constant ($k_{obs}$) can be derived as in Eq. 8 assuming that $[L] = [L_T]$ is constant with time.

$$k_{obs} = \frac{k_0 + k_1K_{1:1}[L] + k_2K_{1:1}K_{1:2}[L]^2}{1 + K_{1:1}[L] + K_{1:1}K_{1:2}[L]^2} \quad (8)$$

The values for $k_1$, $k_2$, $K_{1:1}$, and $K_{1:2}$ can be estimated from stability data by nonlinear regression of Eq. 8. The values for degradation rate constants ($k_1$, $k_2$) and stability constants ($K_{1:1}$, $K_{1:2}$) for prodrug B are shown in Table I. The results show that degradation of the prodrug B is slower in a 1:2 complex than in a 1:1 complex. The observed stability constants from stability data are in good agreement with the stability constants obtained from the phase-solubility study.

The stability of the prodrug B at lower temperatures can be estimated from the Arrhenius equation:

Table I. Pseudo-First-Order Rate Constants for Degradation of O,O'-Dicyclobutanecarbonyl (1,2-ethylene) Bispilocarpic Acid Diester at pH 7.4 (40°C–70°C) and Stability Constants of Inclusion Complexes Calculated with Curve Fitting Method from Degradation Data. $k_0$ is the Degradation Rate Constant of Uncomplexed Drug. $k_1$ and $k_2$ Represents the Pseudo-First-Order Rate Constants for Degradation of Drug in 1:1-Complex and 1:2-Complex, Respectively. $K_{1:1}$ and $K_{1:2}$ Represent the Stability Constants of the 1:1-Complex and 1:2 Complex, Respectively

| $T$ (°C) | $k_0 \times 10^4$ ($h^{-1}$) | $k_1 \times 10^4$ ($h^{-1}$) | $k_2 \times 10^4$ ($h^{-1}$) | $K_{1:1}$ ($M^{-1}$) | $K_{1:2}$ ($M^{-1}$) |
|---|---|---|---|---|---|
| 70 | 492.7 | 181.7 | 78.8 | 152.5 | 18.2 |
| 60 | 201.7 | 63.4 | 35.4 | 141.9 | 18.5 |
| 50 | 79.3 | 28.1 | 10.7 | 154.3 | 16.8 |
| 40 | 25.9 | 7.9 | 3.5 | 127.5 | 20.8 |

$$\log k = -\frac{E_a}{2.303\ R} \times \frac{1}{T} + \log A \qquad (9)$$

where, $E_a$ is the apparent energy of activation, $A$ is frequency factor, $R$ is the gas constant and $T$ is the temperature in Kelvins. The Arrhenius plots of the degradation rates of prodrug B in the absence and presence of HP-β-CD over the temperature range of 40°C–70°C formed parallel lines, which shows that the stabilization effect is same over the temperature range studied. Besides, the results indicate that complexation between prodrug B and HP-β-CD is not significantly dependent on temperature, which can be seen from the stability constants $K_{1:1}$ and $K_{1:2}$ in Table I. The half-lives ($t_{1/2}$) of the prodrug B in temperatures of 40°C–70°C and the calculated shelf-lives ($t_{90\%}$) of prodrug B at lower temperatures (25°C–4°C) are summarized in Table II. Based on the Arrhenius plots, the shelf-life of the prodrug B increased from 4 months (in a absence of HP-β-CD) to 26 months (in presence of 72.5 mM HP-β-CD) at 4°C. Table II shows also that it may be possible to increase the stability of prodrug B by increasing cyclodextrin concentration, as evidenced by an increase of stability of prodrug B as a function of cyclodextrin concentration at every temperature studied.

Figure 4 shows concentrations of 1:1 and 1:2 inclusion complexes and uncomplexed prodrug B molecules in a aqueous solutions used in stability studies. The concentrations of 1:1 and 1:2 inclusion complexes were calculated using the equations 10 and 11.

$$[SL] = \frac{K_{1:1}[S_t][L]}{1 + K_{1:1}[L] + K_{1:1}K_{1:2}[L]^2} \qquad (10)$$

$$[SL_2] = K_{1:2}[SL][L] \qquad (11)$$

where ($S_t$) is the initial concentration of prodrug B in stability studies (194.05 μmol/l) and $K_{1:1}$ and $K_{1:2}$ are 143 $M^{-1}$ and 29 $M^{-1}$, respectively. Figure 4 shows that at low HP-β-CD concentrations, most of the drug is in the 1:1-complex form while at high HP-β-CD concentrations the concentration of the 1:2-complex may dominate. These theoretical calculations are in good agreement with experimental stability data, assuming that the prodrug molecules are more stable in the 1:2 complexed form than in the 1:1 complexed form. Figure 4 shows that at the highest cyclodextrin concentration in these stability studies, only 65% of drug molecules are in the 1:2 complex form. Thus it is possible to increase the stability of prodrug B by increasing HP-β-CD concentrations in aqueous solutions.

## Conclusions

In the present study, it was found that HP-β-CD forms both 1:1 and 1:2 inclusion complexes with O,O'-dipivalyl (1,4-xylylene) bispilocarpic acid diester (prodrug A) and O,O'-dicyclobutanecarbonyl (1,2-ethlyene) bispilocarpic acid diester (prodrug B) in aqueous solution at pH 7.4. Complexation of pilocarpine prodrug with HP-β-CD increased the aqueous solubility of prodrugs and the stability of prodrug B. The degradation rate of prodrug B was slower in a 1:2-complex than in a 1:1-complex and the relative amounts 1:1 and 1:2 inclusion complexes was dependent on cyclodextrin concentration. The results suggest that CDs will be useful additives in the solubilization and stabilization of lipophlic ester-prodrugs of pilocarpine.

Table II. Observed Half-Lives ($t_{1/2}$) and Shelf-Lives ($t_{90\%}$) for Overall Degradation of O,O'-Dicyclobutanecarbonyl (1,2-Ethylene) Bispilocarpic Acid Diester at Various Temperatures, in the Presence or Absence of HP-β-CD at pH 7.4

| Temp. (°C) | $t_{1/2}$ (h)/Relative stabilization[a] | | | | | |
|---|---|---|---|---|---|---|
| | 0.0 mM (CD) | 7.3 mM (CD) | 14.5 mM (CD) | 29.0 mM (CD) | 43.5 mM (CD) | 72.5 mM (CD) |
| 70 | 14.1 | 22.6/1.6 | 28.3/2.0 | 35.4/2.5 | 41.8/3.0 | 52.0/3.7 |
| 60 | 34.4 | 55.4/1.6 | 69.7/2.0 | 94.2/2.7 | 108.6/3.2 | 138.6/4.0 |
| 50 | 87.4 | 143.7/1.6 | 173.0/2.0 | 228.2/2.6 | 338.5/3.9 | 362.8/4.2 |
| 40 | 267.1 | 421.1/1.6 | 557.4/2.1 | 740.1/2.8 | 919.2/3.4 | 1217.0/4.6 |
| Temp. (°C) | $t_{90\%}$ (days)/Relative stabilization | | | | | |
| | 0.0 mM (CD) | 7.3 mM (CD) | 14.5 mM (CD) | 29.0 mM (CD) | 43.5 mM (CD) | 72.5 mM (CD) |
| 25[b] | 8.9 | 14.1/1.6 | 18.5/2.1 | 25.2/2.8 | 36.4/4.1 | 45.0/5.1 |
| 15[b] | 30.1 | 47.7/1.6 | 63.5/2.1 | 87.9/2.9 | 133.6/4.4 | 165.7/5.5 |
| 4[b] | 127.8 | 201.4/1.6 | 273.4/2.1 | 385.1/3.0 | 623.0/4.9 | 775.1/6.1 |

[a] $t_{1/2}$ with CD/$t_{1/2}$ without CD.
[b] Calculated value.

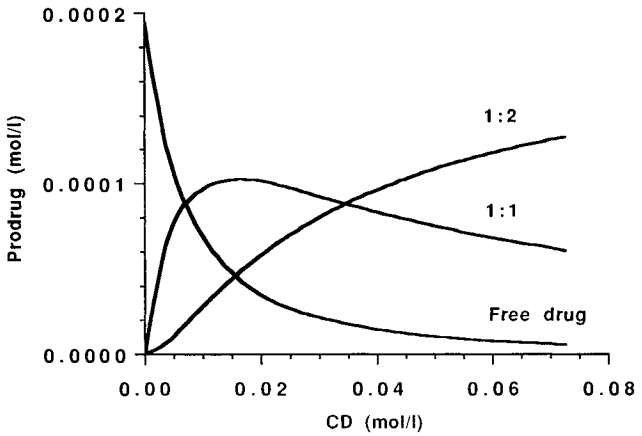



Fig. 4. The calculated concentrations of 1:1-complexes, 1:2-complexes and uncomplexed drug molecules for different HP-β-CD concentrations in stability studies of O,O'-dicyclobutanecarbonyl (1,2-ethylene) bispilocarpic acid diester at pH 7.4.

## ACKNOWLEDGMENTS

Pekka Jarho would like to thank the Pharmaceutical Research Foundation, the Emil Aaltonen Foundation and the Finnish Cultural Foundation for financial support. Arto Urtti and Tomi Järvinen are supported by the Academy of Finland.

## REFERENCES

1. R. Lazare and M. Horlington. Pilocarpine levels in the eyes of rabbits following topical application. *Exp. Eye Res.* **21**:281–287 (1975).
2. H. Bundgaard, E. Falch, C. Larsen, G. L. Mosher, and T. J. Mikkelson. Pilocarpine prodrugs II. Synthesis, stability, bioconversion and physicochemical properties of sequentially labile pilocarpine acid diesters. *J. Pharm. Sci.* 75:775–783 (1986).
3. P. Druzgala, D. Winwood, M. Drewniak-Deyrup, S. Smith, N. Bodor, and J. J. Kaminski. New water-soluble pilocarpine derivatives with enhanced and sustained muscarinic activity. *Pharm. Res.* 9:372–377 (1992).
4. T. Järvinen. Improvement of physicochemical properties and chemical kinetics of pilocarpine by prodrug technique: Ph.D. Thesis, the University of Kuopio, Finland (1992).
5. T. Järvinen, P. Suhonen, A. Urtti, and P. Peura. O,O'-(1,4-xylylene)bispilocarpic acid esters as new potential double prodrugs of pilocarpine for improved ocular delivery. II. Physicochemical properties, stability, solubility and enzymatic hydrolysis. *Int. J. Pharm.* 75:259–269 (1991).
6. P. Suhonen, T. Järvinen, P. Rytkönen, P. Peura, and A. Urtti. Improved corneal pilocarpine permeability with O,O'-(1,4-xylylene)bispilocarpic acid ester double prodrugs. *Pharm. Res.* **8**:1539–1542 (1991).
7. T. Järvinen, K. Järvinen, A. Urtti, D. Thompson, and V. J. Stella. Sulfobutyl ether β-cyclodextrin (SBE-β-CD) in eyedrops improves the tolerability of a topically applied pilocarpine prodrug in rabbits. *J. Ocul. Pharmacol.* (1995) in press.
8. L. A. Wheeler. The use of inclusion complexes of prostaglandins with cyclodextrins in the treatment of ocular hypertension. Eur. Pat., O 435 682 A3, (1991).
9. T. Loftsson, H. Fridriksdóttir, S. Thórisdóttir, E. Stefansson, A. M. Sigurdardóttir, Ö. Gudmundsson, and T. Sigthórsson. 2-hydroxypropyl-β-cyclodextrin in topical carbonic anhydrase inhibitor formulations. *Eur. J. Pharm. Sci.* **1**:175–180 (1994).
10. A. Usayapant, A. H. Karara, and M. M. Narurkar. Effect of 2-hydroxypropyl-β-cyclodextrin on the ocular absorption of dexamethasone and dexamethasone acetate. *Pharm. Res.* **8**:1495–1499 (1991).
11. T. Loftsson, H. Fridriksdóttir, S. Thórisdóttir, and E. Stefansson. The effect of hydroxypropyl methylcellulose on the release of dexamethasone from aqueous 2-hydroxypropyl-β-cyclodextrin formulations. *Int. J. Pharm.* **104**:181–184 (1994).
12. O. Reer, T. K. Bock, and B. W. Müller. In vitro corneal permeability of diclofenac sodium in formulations containing cyclodextrins compared to the commercial product voltaren ophtha. *J. Pharm. Sci.* 83:1345–1349 (1994).
13. K. A. Freedman, J. W. Klein, and C. E. Crosson. Beta-cyclodextrins enhance bioavailability of pilocarpine. *Curr. Eye Res.* **12**:641–647 (1993).
14. K. Järvinen, T. Järvinen, D. O. Thompson, and V. J. Stella. The effect of a modified β-cyclodextrin, SBE4-β-CD, on the aqueous stability and ocular absorption of pilocarpine. *Curr. Eye. Res.* **13**:891–905 (1994).
15. H. Doorne. Interactions between cyclodextrins and ophthalmic drugs. *Eur. J. Pharm. Biopharm.* 39:133–139 (1993).
16. T. Järvinen, P. Suhonen, S. Auriola, J. Vepsäläinen, A. Urtti, and P. Peura. O,O'-(1,4-xylylene) bispilocarpic acid esters as new potential double prodrugs of pilocarpine for improved ocular delivery. I. Synthesis and analysis. *Int. J. Pharm.* 75:249–258 (1991).
17. T. Higuchi and K. A. Connors. Phase-solubility techniques. *Adv. Anal. Chem. Instrum.* **4**:117–212 (1965).