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Sulfobutyl Ether β-Cyclodextrin (SBE-β-CD) in Eyedrops Improves the Tolerability of a Topically Applied Pilocarpine Prodrug in Rabbits

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ABSTRACT

The effects of a novel, modified β-cyclodextrin (SBE4-β-CD; a variably substituted sulfobutyl ether with an average degree of substitution of four) on eye irritation and miotic response of an ophthalmically applied pilocarpine prodrug, O,O'-dipropionyl-(1,4-xylylene) bispilocarpate, in albino rabbits were studied. Compared to the commercial pilocarpine eyedrop solution (163 mM, equivalent to 3.4% pilocarpine), 12 - 24 mM pilocarpine prodrug solutions (equivalent to 0.5 - 1.0% pilocarpine, respectively) decreased peak miotic intensity (I_{max}) and increased the time to reach peak (t_{max}), but did not significantly affect values for the area under the miosis versus time curves (AUC), i.e. 12 - 24 mM pilocarpine prodrug appeared to be equivalent to 163 mM pilocarpine. Ocularly applied 12 - 24 mM pilocarpine prodrug solutions, however, were more irritating than a commercial pilocarpine eyedrop solution. Coadministered SBE4-β-CD significantly decreased the eye irritation of the pilocarpine prodrug solutions. Coadministered SBE4-β-CD did not affect the miotic response of prodrug solution when the molar ratio of SBE4-β-CD to prodrug was low. However, increasing the molar ratio of SBE4-β-CD to prodrug decreased the I_{max} and AUC values. The results show that eye irritation of the pilocarpine prodrug is prevented by levels of SBE4-β-CD that do not affect the apparent ocular absorption of the prodrug.

INTRODUCTION

Pilocarpine is a widely used drug for controlling the elevated intraocular pressure associated with glaucoma. However, duration of action for ophthalmic pilocarpine is short and its ocular bioavailability is only 0.1-3% of the instilled pilocarpine dose (1-3). Thus, the prodrug technique has been applied to improve the ocular delivery of pilocarpine (4-6). O,O'-Dipropionyl-(1,4-xylylene) bispilocarpate (Fig. 1a) is a dimeric pilocarpine double prodrug which releases pilocarpine via enzymatic and chemical hydrolysis in the eye. Due to its higher lipophilicity, the corneal permeability coefficient for O,O'-dipropionyl-(1,4-xylylene) bispilocarpate is several times higher than that for pilocarpine (7) and ocular absorption of pilocarpine is increased by this pilocarpine prodrug (8). Unfortunately, preliminary results suggest that the clinical acceptability of O,O'-dipropionyl-(1,4-xylylene) bispilocarpate may be mostly limited by the eye irritation induced by the pilocarpine prodrug solution. For stability and solubility reasons, the pH of O,O'-dipropionyl-(1,4-xylylene) bispilocarpate eyedrops cannot be higher than 5.0 (6). Acidic eyedrop solutions may result in decreased ocular bioavailability due to the resulting induced lacrimation and lower corneal permeability (9).

Cyclodextrins (CDs) are a group of homologous cyclic oligosaccharides consisting of six, seven or eight glucose units called α-, β- or γ-cyclodextrin, respectively. CDs are well-known for their ability to form inclusion complexes with a wide variety of hydrophobic drugs. Inclusion complex formation between drug and CD may improve drug solubility and/or stability (10-12) as well as bioavailability of the drug (13, 14). The use of CDs has been studied extensively in parenteral, oral, rectal and dermal formulations (15-17), however, the effects of CDs on ocular delivery of topically applied drugs are poorly understood. It has been reported that coadministration of hydroxypropyl-β-CD increases the intraocular pressure lowering effect of prostaglandins (18) and ocular absorption of dexamethasone, dexamethasone acetate (19), and pilocarpine (20).

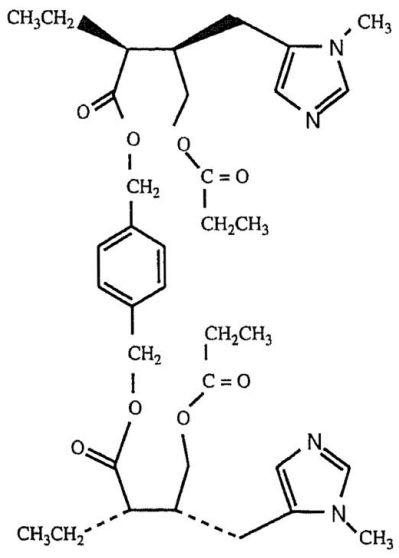
SBE4-β-CD and SBE7-β-CD (average degree of butylsulfonate substitute of seven) have been developed with the purpose of improving the toxicity, aqueous solubility and subsequent pharmaceutical usefulness of β-CD (21). In SBE-β-CD, the sulfonate group (as its sodium salt) is spaced from the cyclodextrin cavity by a butyl chain to ensure that the new substituents do not interfere with the inclusion process. The degree of substitution indicates the number of sulfobutyl moieties per cyclodextrin molecule. The solubility enhancements observed with SBE4-β-CD (Fig. 1b) are comparable to or higher than those observed with hydroxypropyl-β-CD (22, 23).

The purpose of this study was to evaluate the effect of SBE4-β-CD on the ocular absorption, as indicated by miosis, and eye irritation of bispilocarpic acid diester prodrug. This study demonstrates the usefulness of this new β-cyclodextrin derivative as an adjuvant in lipophilic prodrug eyedrop solutions.

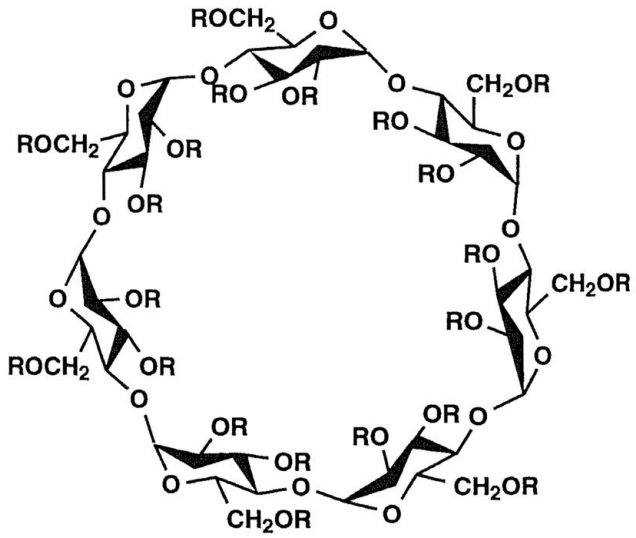
MATERIALS AND METHODS

Solution Preparation

The synthesis and identification procedures for pilocarpine prodrug, O,O'-dipropionyl-(1,4-xylylene) bispilocarpate as a fumarate salt and for SBE4-β-CD (average mw = 1725.9) have been described previously (23-25). The chemical structures for O,O'-dipropionyl-(1,4-xylylene) bispilocarpate and SBE4-β-CD are shown in Fig. 1.



a



R = $CH_2CH_2CH_2CH_2SO_3Na$ or H (average degree of sulfobutyl substitution is four).

b

FIGURE 1. The Chemical Structure of O,O'-Dipropionyl (1,4-Xylylene) Bispilocarpate (a) and Tetra-Sulfobutyl Ether β-Cyclodextrin (SBE4-β-CD) (b).

Pilocarpine in eyedrop formulation (Oftan Pilocarpin®, 163 mM) was purchased from Leiras Oy (Finland). Pilocarpine eyedrops (pH = 5.0) contained pilocarpine hydrochloride 40 mg/mL and

benzalkonium chloride 40 μg/mL. Pilocarpine prodrug and pilocarpine prodrug containing SBE4-β-CD solutions were prepared by dissolving the required amount of pilocarpine prodrug and SBE4-β-CD in 5.0 mL of double distilled water, and pH of the solutions was adjusted to 5.0 or 6.0 with sodium hydroxide. The solutions were made isotonic with sodium chloride. The pilocarpine prodrug solutions were prepared equivalent to a 0.5% and a 1.0% pilocarpine solution.

The control solutions were 0.9% sodium chloride solution (Sigma Chemical Co. St. Louis, MO, pH was adjusted to 5.0 with hydrochloride acid) and 10% (58 mM) SBE4-β-CD solution (pH 5.0). The latter solution was prepared as described above but no prodrug was dissolved in solution.

Animals

The experimental animals used in this study were adult male New Zealand White albino rabbits weighing between 2.8 - 3.6 kg. The rabbits were housed singly in cages under standard laboratory conditions: 12 h dark/12 h light cycle. The rabbits were given food and water *ad libitum*. The experiments conformed to the ARVO Resolution on the use of animals in research.

Evaluation of Eye Irritation

To estimate the discomfort caused by an instilled eyedrop, the extent of eyelid closure (closed or half-closed) following immediately after unilateral eyedrop administration (25 μL) was observed and recorded. Once the rabbit opened its eye fully, the eyelid closure behavior was no longer recorded. The amount of mucoidal discharge at 0.25 and 1 h after eyedrop administration was also recorded. Mucoidal discharge was scored from 0 to 2 as follows: 0 = normal, no lacrimation, 1 = slight discharge (any amount different from normal, clear discharge) and 2 = strong discharge (moisten the lids and hairs just adjacent to the lids, milk-like discharge). Eye irritation was always evaluated by the same operator who did not know composition of an instilled solution. At least 72 h wash-out time was allowed for each rabbit between dosings.

Miotic Response

To perform each evaluation, the rabbit was placed in a plastic restraining box located in a quiet room with constant light condition. The rabbit was kept in that environment for 1 h before eyedrop administration in order to acclimatize it to the environment. The test solution (25 μL) was instilled on the upper corneoscleral limbus. During the instillation the upper eyelid was slightly pulled away from the globe. All solutions were administered into the right eye while the left eye remained untreated. To measure the pupil diameter, the eyes were photographed from a constant distance at 0.5, 0.25 and 0 h before and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 6 h after drug administration. The negatives were enlarged with a microfilm reflector and the pupillary diameters were determined as a mean of horizontal and vertical diameters. Pupillary diameter at the time of eyedrop administration (at 0 h) was used as a baseline pupillary diameter. The lighting conditions in laboratory gave a baseline pupillary diameter in the range of 6.4 - 7.9 mm. The studies were set up in a 5 x 5 or a 6 x 6 masked cross-over design. At least 72 h wash-out time was allowed for each rabbit between dosings.

Analysis of Data

The magnitude of the miotic responses (%) was calculated as $((PD_0 - PD_t)/PD_0) \times 100\%$, where PD_0 is a baseline pupillary diameter and PD_t is pupillary diameter at the time point. For each rabbit at each time point, individual miotic response values for 0.9% NaCl solution were subtracted from the miotic response values for drug solution and then the following pharmacokinetic parameters were calculated. Areas under the miotic response versus time curves ($AUC_{0\text{-}6h}$) were calculated using the trapezoidal method (26), and the value for miotic response was assumed to be equal to zero if it was negative after subtraction of the value for 0.9% NaCl solution at the same time point. Peak miosis intensity (I_{max}) and its time (t_{max}) were determined from the actual data points. The duration of response was determined from miosis versus time profiles by calculating the total period of time when miosis was equal or more than 30%, 10% or 5%. All the values are expressed as mean ± S.E. (standard error). A one-factor analysis of variance (ANOVA for repeated measurements) was used to test the statistical significance of differences between groups; significance in the differences in the means was tested using Fisher's Protected Least Significant Difference (PLSD) at 95% confidence.

RESULTS

Eye Irritation

Eyelid closure data are shown in Fig. 2 (eye closed or half-closed) and in Table 1 (sum of eye lid closure). Compared to a commercial pilocarpine eyedrop solution, Oftan Pilocarpin® (163 mM, 3.4% pilocarpine), duration of eyelid closure after administration of 24 mM prodrug solution (equivalent to a 1.0 % pilocarpine solution) was significantly longer ($p<0.05$, by Fisher's PLSD test). The rabbits kept their eyes closed or half-closed (sum of eye lid closure) for 1.2 ± 0.3 min, for 2.1 ± 0.6 min and for 11.5 ± 5.6 min after ocular administration of a 163 mM commercial pilocarpine solution, 12 mM prodrug solution and 24 mM prodrug solution, respectively (Table 1). Prodrug solutions caused significantly stronger ($p<0.05$, by Fisher's PLSD test) ocular discharge at 0.25 h (scores were 0.8 ± 0.3 for 24 mM prodrug and 0.3 ± 0.2 for 12 mM prodrug) and at 1 h (scores were 1.8 ± 0.2 for 24 mM prodrug and 0.7 ± 0.3 for 12 mM prodrug) after ocular administration than 163 mM commercial pilocarpine solution (0.0 ± 0.0).

TABLE 1

Miotic Activity Parameters and Duration of Eyelid Closure After Ocular Administration of 25 µL of Pilocarpine or Pilocarpine Prodrug (O,O'-Dipropionyl-(1,4-Xylylene) Bispilocarpate) in the Presence or Absence of Tetra-Sulfobutyl Ether β-Cyclodextrin (SBE4-β-CD) in Albino Rabbits (mean ± S.E., n = 5-6).

Solution	pH	Peak time[a] (min)	I_{max}[b] (%)	Eye lid closure[c] (min)
Oftan Pilocarpin® (163 mM, 3.4%)	5.0	21 ± 3	36.1 ± 1.9	1.2 ± 0.3
12 mM Pilocarpine Prodrug (equivalent to 0.5% pilocarpine)	5.0	132 ± 28*	17.5 ± 2.2*	2.1 ± 0.6
12 mM Pilocarpine Prodrug + 2.5% (14.5 mM) SBE4-β-CD	5.0	114 ± 35*	16.9 ± 2.4*	1.0 ± 0.4
12 mM Pilocarpine Prodrug + 10 % (58 mM) SBE4-β-CD	5.0	150 ± 21*	12.5 ± 1.2*	0.5 ± 0.3‡
24 mM Pilocarpine Prodrug (equivalent to 1% Pilocarpine)	5.0	118 ± 23*	21.9 ± 4.8*	11.5 ± 5.6*
24 mM Pilocarpine Prodrug + 5% (29 mM) SBE4-β-CD	5.0	103 ± 27*	20.3 ± 2.1*	1.5 ± 0.3#
24 mM Pilocarpine Prodrug + 10 % (58 mM) SBE4-β-CD	5.0	118 ± 23*	16.8 ± 1.5*	0.8 ± 0.2#
24 mM Pilocarpine Prodrug + 5% (29 mM) SBE4-β-CD	6.0	123 ± 24*	22.5 ± 1.6*	1.9 ± 0.5#
24 mM Pilocarpine Prodrug+ 10 % (58 mM) SBE4-β-CD	6.0	135 ± 23*	17.7 ± 2.9*	0.8 ± 0.3#

[a] Time to reach I_{max}, [b]Maximum miotic effect, [c]Period of time when eye was closed or half-closed.

* Significantly different from the value for Oftan Pilocarpin® (163 mM) ($p<0.05$, by Fisher's PLSD test).

\# Significantly different from the value for 24 mM prodrug ($p<0.05$, by Fisher's PLSD test).

‡ Significantly different from the value for 12 mM prodrug ($p<0.05$, by Fisher's PLSD test).

The duration of eyelid closure after ocular administration of isotonic, non-drug containing SBE4-β-CD solution (10 % w/v, 58 mM) was similar to that for 0.9% sodium chloride solution (Fig. 2). The duration of eyelid closure after topical administration of 24 mM pilocarpine prodrug solution was decreased significantly ($p < 0.05$, by Fisher's PLSD test) by SBE4-β-CD coadministration (29-58 mM) (Table 1, Fig. 2). The rabbits kept their eyes closed or half-closed (sum of eyelid closure) for 0.8 -1.9 min after ocular administration of the pilocarpine prodrug (24 mM) containing SBE4-β-CD (29 - 58 mM) solutions. In addition, discharge observed at 0.25 and 1h after ocular administration of 12 mM and 24 mM pilocarpine prodrug solutions was eliminated by SBE4-β-CD coadministration. These results show that eye irritation caused by 12 - 24 mM pilocarpine prodrug solution that contains 14.5 - 58 mM of SBE4-β-CD is comparable to the eye irritation caused by 163 mM commercial pilocarpine eyedrops (Fig. 2, Table 1). Increasing the pH of pilocarpine prodrug containing SBE4-β-CD solution from 5.0 to 6.0 did not affect the eye irritation due to solution (Fig. 2, Table 1).

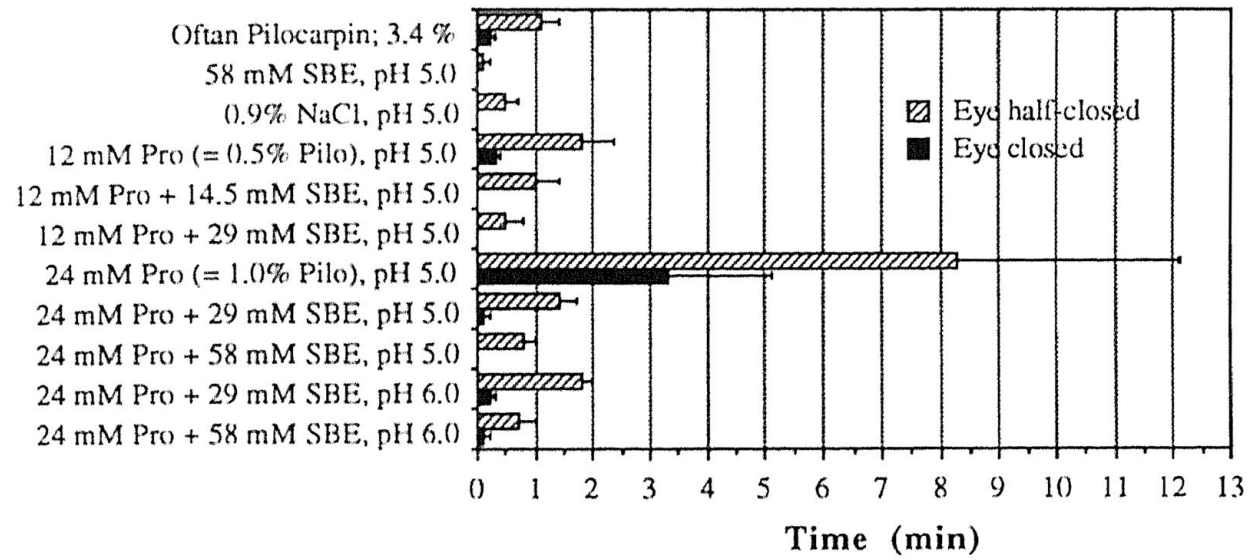


FIGURE 2. Duration of Eyelid Closure after Ocular Administration of 25 µL of Pilocarpine (Oftan Pilocarpin®) or Pilocarpine Prodrug (O,O'-Dipropionyl-(1,4-Xylylene) Bispilocarpate) (PRO) in the Presence or Absence of SBE4-β-CD (SBE) in Albino Rabbits (mean ± S.E., n = 5-6).

Miotic Response

The results for miosis studies are summarized in Tables 1 and 2. Miosis versus time profiles for 0.9% sodium chloride and isotonic 58 mM SBE4-β-CD solutions were similar (Fig. 3).

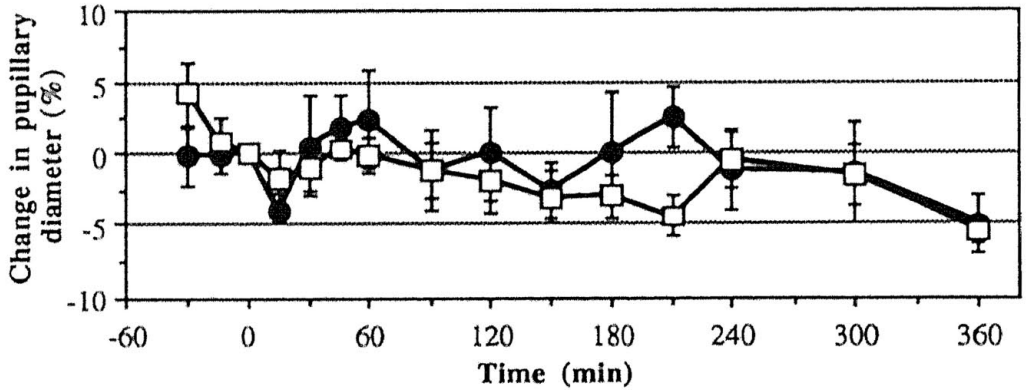


FIGURE 3. Change in Pupillary Diameter after Ocular Administration of 25 µL of 0.9% NaCl (□) or 58 mM (10% w/v) SBE4-β-CD (●) in Albino Rabbits. Values are means ± S.E. (n = 5-6).

Compared with 163 mM commercial pilocarpine eyedrops (3.4% pilocarpine), 12 and 24 mM prodrug solutions (equivalent to a 0.5% and a 1.0 % pilocarpine solution, respectively) decreased peak miotic intensity (I_{max}) and increased the time to reach peak (t_{max}) significantly ($p< 0.05$, by Fisher's PLSD test). However, 12 and 24 mM prodrug solutions had values for areas under the miotic response versus time curves (AUC_{0-6h}) and the period of time when miotic response was equal or more than 5% or 10% comparable to the 163 mM pilocarpine solution (Table 2, Fig. 4).

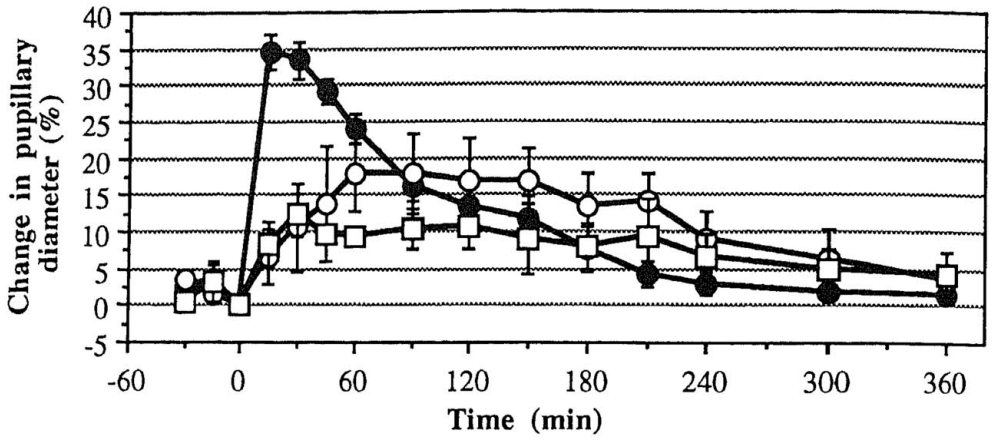


FIGURE 4. Change in Pupillary Diameter after Ocular Administration of 25 µL of Pilocarpine (163 mM, 3.4%) (●) or Pilocarpine Prodrug (24 mM, Equivalent to 1.0% Pilocarpine (O) and 12 mM, Equivalent to 0.5% Pilocarpine (□)) Solutions at pH 5.0 in Albino Rabbits. Values are means ± S.E.; n = 5 - 6.

The miotic response of 12 mM pilocarpine prodrug and pilocarpine prodrug (12 mM) containing SBE4-β-CD (2.5% (w/v), 14.5 mM) solutions was similar as indicated by similar t_{max}, I_{max} and AUC_{0-6h} values (Tables 1 and 2). Compared to a 12 mM prodrug solution, prodrug (12 mM) containing SBE4-β-CD (10% (w/v), 58 mM) solution did not significantly affect t_{max} and I_{max} values but significantly decreased AUC_{0-6h} value and the period of time when miotic response was more than 10% ($p< 0.05$, by Fisher's PLSD test) (Tables 1 and 2, Fig. 5).

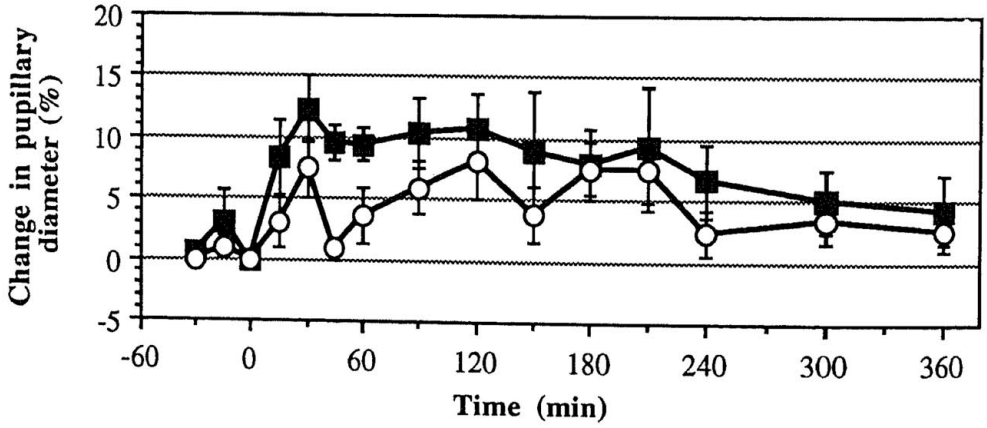


FIGURE 5. Change in Pupillary Diameter after Ocular Administration of 25 µL of Pilocarpine Prodrug (12 mM) (■) or Pilocarpine Prodrug (12 mM) Containing SBE4-β-CD (58 mM, 10% w/v) (O) Solution in Albino Rabbits. Values are means ± S.E.; n = 5.

In the case of the 24 mM pilocarpine prodrug solutions (pH 5.0 - 6.0), coadministration of 29 - 58 mM of SBE4-β-CD in solutions did not significantly change the miotic activity parameters (t_{max}, I_{max}, AUC_{0-6h}, duration time) (Tables 1 and 2). However, increasing the concentration of SBE4-β-CD in 24 mM pilocarpine prodrug solution did tend to reduce the miotic response of the solution.

TABLE 2

Miotic Activity Parameters after Administration of 25 μL of Pilocarpine or Pilocarpine Prodrug (O,O'-Dipropionyl-(1,4-Xylylene) Bispilocarpate) in the Presence or Absence of Tetra-Sulfobutyl Ether β-Cyclodextrin (SBE4-β-CD) in Albino Rabbits (mean ± S.E., n = 5-6).

Solution	pH	Duration 5%[a] (min)	Duration 10%[b] (min)	Duration 30%[c] (min)	$AUC_{0-6\ h}$[d] (% x h)
Oftan Pilocarpin® (163 mM, 3.4 %)	5.0	206 ± 20	143 ± 13	27 ± 7	66.5 ± 4.4
12 mM Prodrug (resp. 0.5% Pilocarpine)	5.0	219 ± 22	124 ± 41	0	46.3 ± 9.0
12 mM Prodrug + 2.5% (14.5 mM) SBE4-β-CD	5.0	254 ± 36	149 ± 49	0	52.4 ± 11.7
12 mM Prodrug + 10 % (58 mM) SBE4-β-CD	5.0	154 ± 26#	34 ± 20*‡#	0	27.2 ± 4.5*‡#
24 mM Prodrug (Resp. 1% Pilocarpine)	5.0	243 ± 34	161 ± 49	31 ± 25	69.0 ± 23.4
24 mM Prodrug + 5% (29 mM) SBE4-β-CD	5.0	263 ± 35	169 ± 38	0	59.1 ± 9.9
24 mM Prodrug + 10 % (58 mM) SBE4-β-CD	5.0	231 ± 25	101 ± 22	0	43.2 ± 4.9
24 mM Prodrug + 5% (29 mM) SBE4-β-CD	6.0	288 ± 26*	184 ± 21	0	67.1 ± 6.4
24 mM Prodrug + 10 % (58 mM) SBE4-β-CD	6.0	214 ± 38	98 ± 34	0	43.9 ± 9.4

[a-c]Time period when miosis was ≥ 5%[a], ≥ 10%[b] and ≥ 30%[c]; [d]Area under miosis versus time curve.

* Significantly different from the value for Oftan Pilocarpin® (163 mM) ($p<0.05$, by Fisher's PLSD test).

‡ Significantly different from the value for 12 mM prodrug ($p<0.05$, by Fisher's PLSD test).

\# Significantly different from the value for 12 mM prodrug + 2.5% SBE4-β-CD ($p<0.05$, by Fisher's PLSD test).

Coadministered SBE4-β-CD increased the aqueous solubility of the prodrug as indicated by the following results: For solubility reasons, it was not possible to prepare 24 mM pilocarpine prodrug solution at pH values higher than 5.0. However, 24 mM pilocarpine prodrug solutions could be made at pH 6.0 by coadministering SBE4-β-CD in the solution. Increasing the pH of the prodrug (24 mM) containing SBE4-β-CD (29-58 mM) solutions from 5.0 to 6.0 did not affect miotic activity parameters of the solutions significantly (Tables 1 and 2).

The results show that the eye irritation of ophthalmic pilocarpine prodrug, O,O'-dipropionyl-(1,4-xylylene) bispilocarpate, is prevented by levels of SBE4-β-CD (Fig. 6a) that do not affect the apparent ocular delivery of the prodrug in rabbits (Fig. 6b).

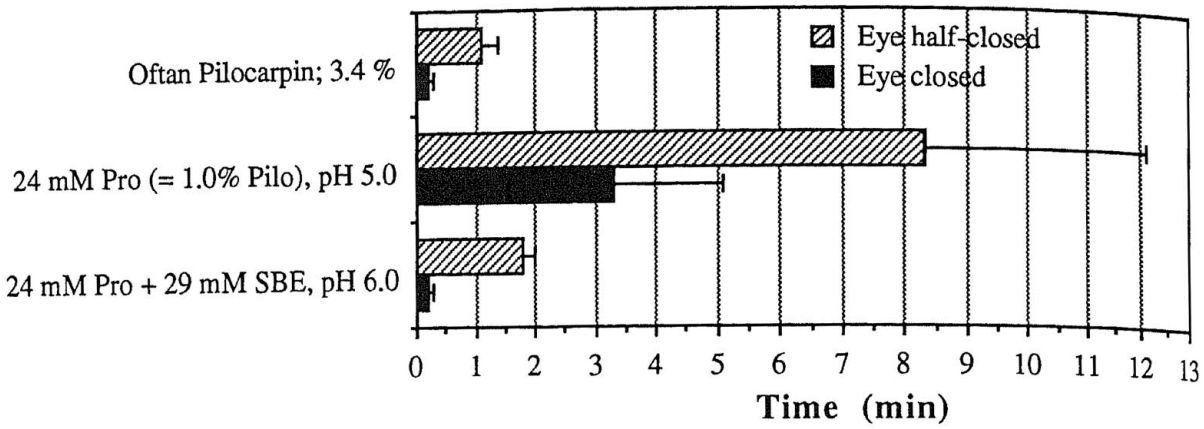


FIGURE 6a. Duration of Eyelid Closure after Ocular Administration of 25 μL of Pilocarpine (Oftan Pilocarpin®; 163 mM), 24 mM Pilocarpine Prodrug (pH = 5.0) and 24 mM Pilocarpine Prodrug Containing 29 mM (5% w/v) SBE4-β-CD (pH = 6.0) Solution in Albino Rabbits. Values are means ± S.E.; n = 6.

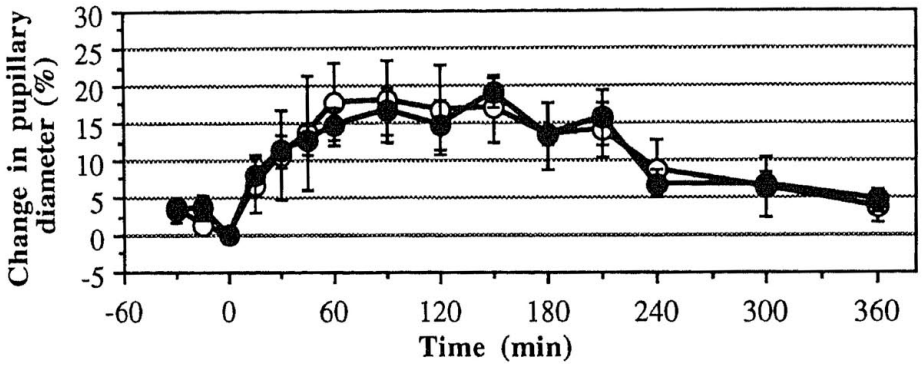


FIGURE 6b. Change in Pupillary Diameter after Ocular Administration of 25 μL of 24 mM Pilocarpine Prodrug Solution (pH 5.0) (O) or 24 mM Pilocarpine Prodrug with 29 mM (5% w/v) SBE4-β-CD Solution (pH 6.0) (●) in Albino Rabbits. Values are means ± S.E.; n = 6.

DISCUSSION

Eye Irritation

SBE4-β-CD itself was not irritating when given topically in rabbit eyes and consequently, it might be a suitable adjuvant for ophthalmic drug preparations. The maximum strength of pilocarpine eyedrops that are in common use among glaucoma patients is 163 mM (equivalent to 4% pilocarpine hydrochloride). Consequently, the eye irritation of a topically applied pilocarpine prodrug should not be more than that seen with 163 mM pilocarpine eyedrops. In the present study, however, a new pilocarpine prodrug (24 mM) applied topically caused significantly more eye irritation than 163 mM pilocarpine eyedrops. This might be due to a rapid absorption of lipophilic prodrug molecules into the lipophilic corneal epithelium and/or precipitation of prodrug molecules in the precorneal area and/or direct and specific toxicity of this pilocarpine prodrug molecule. Due to the rapid absorption of lipophilic pilocarpine prodrug into the corneal epithelium, temporary high and irritating prodrug concentrations may occur in the epithelium after ocular administration.

Increased aqueous solubility of the pilocarpine prodrug in the presence of SBE4-β-CD suggests that SBE4-β-CD did indeed interact with the pilocarpine prodrug studied. In general, cyclodextrins are well-known for their ability to form inclusion complexes with hydrophobic drugs (15). Drug/β-CD inclusion complex and β-CD itself do not penetrate across biological membranes

largely due to the hydrophilic character of CDs (27, 28). Thus, drug/β-CD complexes must dissociate before drug absorption. Based on these findings, we assume that the pilocarpine prodrug must be released from pilocarpine prodrug/SBE4-β-CD inclusion complexes before ocular delivery of the prodrug can occur. Consequently, coadministered SBE4-β-CD may act to temporarily slow the ocular absorption rate of the pilocarpine prodrug. As a result, coadministered SBE4-β-CD decreased the high initial concentration of the pilocarpine prodrug in the corneal epithelium and thus, decreased eye irritation (Fig. 2).

Eye irritation observed after topical administration of pilocarpine prodrug solutions might also be due to the precipitation of the pilocarpine prodrug in the precorneal fluids. It is known that the pH of instilled solutions will be readjusted to the normal tear pH (7.4 ± 0.1) within a few minutes after administration of an acidic unbuffered eyedrop solution in the rabbit eye (29, 30). The aqueous solubility of the studied pilocarpine prodrug is very poor at pH 7 (< 1 mg/mL) (6). Thus, due to an increase in pH of solution, precipitation of topically applied pilocarpine prodrug molecules at the precorneal area may occur causing eye irritation. The increased aqueous solubility of the pilocarpine prodrug in the presence of SBE4-β-CD may hinder the precipitation of this prodrug in the precorneal fluids and subsequently decrease the eye irritation. The pH of the pilocarpine prodrug/SBE4-β-CD combinations was increased from 5.0 to 6.0 in order to reduce any eye irritation of solutions associated with the use of low pH value. However, eye irritation of the pilocarpine prodrug/SBE4-β-CD combination was not affected by the pH change. This result suggests that the principal reason for eye irritation following administration of the combination solutions is the pilocarpine prodrug molecule itself, not necessarily the acidic pH of the solution.

Miotic Response

Miotic response is widely used as an indication of biological response for ophthalmic pilocarpine (31, 32). Although the precise relationship between pilocarpine induced miosis and its ability to lower intraocular pressure has not been established in rabbits, it has been shown that a linear relationship exits between the area under the miosis versus time curve and aqueous humor concentration of pilocarpine in albino rabbits (33). This is true when pilocarpine concentration in eyedrops is not high enough to cause the absolute maximum miotic response in rabbit eye. Ocularly applied non-drug containing SBE4-β-CD solution did not cause miosis or mydriasis (Fig. 3) and thus, the effect of coadministered SBE4-β-CD on ocular absorption of pilocarpine prodrug was possible to evaluate by the miotic method.

Pilocarpine prodrugs have been developed in order to improve the ocular delivery of pilocarpine, e.g. to eliminate the high peak concentrations of pilocarpine in the eye and to prolong its duration of action (4-6). In the present study, administration of 14.3% (1/7) of the pilocarpine dose as a prodrug (O,O'-dipropionyl-(1,4-xylylene) bispilocarpate) decreased the peak miotic intensity of the solution and increased the time to reach the peak, but did not significantly affect values for the area under the miosis versus time curves (AUC), i.e. 12 mM pilocarpine prodrug (equivalent to 0.5% pilocarpine) appeared to be equivalent to 163 mM (3.4%) pilocarpine. The increased bioavailability of pilocarpine (log P = 0.01, octanol/buffer, pH 7.4) when given as the studied prodrug was presumably due to the higher lipophilicity of the prodrug (log P = 4.08, octanol/buffer, pH 7.4, (6)). The optimum apparent partition coefficient (log P octanol/buffer, pH 7.4) for corneal absorption of a stable drug is 2-3 (34-36). In the case of double prodrugs, it is more complicated to estimate the optimal log P value for corneal permeability because their corneal permeability is dependent both on the lipophilicity of the double prodrug/prodrug/parent drug and on the conversion rate of double prodrug/prodrug to the parent drug. The corneal uptake studies suggest that the largest ocular bioavailability for pilocarpine double prodrugs may be obtained with a larger value of log P (octanol/buffer, pH 7.4) than 2-3 (7, 37).

The effect of coadministered SBE4-β-CD on the miotic response of the pilocarpine prodrug was dependent on the molar ratio of the cyclodextrin to the prodrug. Coadministered SBE4-β-CD did not affect the miotic response of pilocarpine prodrug solution when the molar ratio of cyclodextrin to prodrug was low enough. This result suggests that the rate of dissociation of pilocarpine prodrug/SBE4-β-CD complex in the precorneal area was faster than the rate of ocular absorption of pilocarpine prodrug. However, coadministration of SBE4-β-CD at high concentrations reduced the miotic response of the pilocarpine prodrug solution. As discussed earlier, it is generally assumed that only free drug, not drug/cyclodextrin complex, can penetrate across biological membranes (27, 28, 38). Consequently, substantial complexation of pilocarpine prodrug with SBE4-β-CD can be expected to decrease the ocular delivery of pilocarpine prodrug due to a significant decrease in fraction of free pilocarpine prodrug in solution. Besides, in contrast to oral or parenteral administration, a significant decrease in the fraction of complexed prodrug due to dilution does not occur in the precorneal area after ocular administration because tear fluid volume is very small (≈7 μL).

The pilocarpine prodrug studied is a base with pK_a of 6.7 (6). As the pH of this pilocarpine prodrug solution is increased from 5.0 to 6.0, the fraction of unionized prodrug increases. Because the unionized form of pilocarpine is absorbed across the cornea more easily than its ionized form (39), ocular absorption of the studied pilocarpine prodrug can be expected to increase by increasing pH of the prodrug solution. However, changes in the pH of the pilocarpine prodrug/SBE4-β-CD combination did not affect the miotic response of the solution significantly. This result can be attributed to two factors. Due to the low buffer capacity of the solutions, both pH 5.0 and pH 6.0 solutions were probably readjusted to physiological pH within few minutes after ocular administration (29, 30). On the other hand, the stability constant of the prodrug with SBE4-β-CD may be larger at pH 6.0 than at pH 5.0 due to increased fraction of unionized prodrug in pH 6.0 solution (40) and thus, dissociation of the pilocarpine prodrug/SBE4-β-CD complex may be weaker for the pH 6.0 solution. This could compensate for the positive effect of increased fraction of unionized prodrug in pH 6.0 solution on miotic response.

It can be concluded that eye irritation due to the ophthalmic pilocarpine prodrug, O,O'-dipropionyl-(1,4-xylylene) bispilocarpate, can be prevented by levels of SBE4-β-CD that do not affect the apparent ocular absorption of the pilocarpine prodrug. SBE4-β-CD was not irritating when given topically in the eyes of rabbits; and based on the preliminary results (22), systemically administered SBE4-β-CD seems to be safer than a parent β-cyclodextrin and some other β-cyclodextrin derivatives. Consequently, SBE4-β-CD may be a useful adjuvant in the development of ophthalmic formulations.

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REFERENCES

1. Asseff, C.F., Weisman, R.L., Podos, S.M. and Becker, B. Ocular penetration of pilocarpine in primates. Am. J. Ophthalmol., 75: 212-215, 1973.

2. Chrai, S.S. and Robinson, J.R. Corneal permeation of topical pilocarpine nitrate in the rabbit. Am. J. Ophthalmol., 77: 735-739, 1974.

3. Lazare, R. and Horlington, M. Pilocarpine levels in the eyes of rabbits following topical application. Exp. Eye Res., 21: 281-287, 1975.

4. Bundgaard, H., Falch, E., Larsen, C., Mosher, G.L. and Mikkelson, T.J. Pilocarpine prodrugs II. Synthesis, stability, bioconversion and physicochemical properties of sequentially labile pilocarpine acid diesters. J. Pharm. Sci., 75: 775-783, 1986.

5. Druzgala, P., Winwood, D., Drewniak-Deyrup, M., Smith, S., Bodor, N. and Kaminski, J.J. New water-soluble pilocarpine derivatives with enhanced and sustained muscarinic activity. Pharm. Res., 9: 372-377, 1992.

6. Järvinen, T., Suhonen, P., Urtti, A. and Peura, P. 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.

7. Suhonen, P., Järvinen, T., Rytkönen, P., Peura, P. and Urtti, A. Improved corneal pilocarpine permeability with O,O'-(1,4-xylylene) bispilocarpic acid diester double prodrugs. Pharm. Res., 8: 1539-1542, 1991.

8. Suhonen, P., Järvinen, T., Peura, P., Alaranta, S., Oksala, O. and Urtti, A. Ocular absorption of O,O'-dipropionyl (1,4-xylylene) bispilocarpate, prodrug of pilocarpine, into rabbit eye. In Proceeding of the Symposium on Methods to Overcome Biological Barriers in Drug Delivery, Kuopio University Publications A. Pharmaceutical Sciences 10, Urtti, A., ed. Kuopio University Printing Office, Kuopio, 1993, p. 99.

9. Lee, V.H.L. and Robinson, J.R. Review: topical ocular drug delivery recent developments and future challenges. J. Ocular Pharmacol., 2: 67-108, 1986.

10. Backensfeld, T., Müller, B.W., Wiese, M. and Seydel, J.K. Effect of cyclodextrin derivatives on indomethacin stability in aqueous solution. Pharm. Res., 7: 484-490, 1990.

11. Loftsson, T., Björndóttir, S., Pálsdóttir, G. and Bodor, N. The effects of 2-hydroxypropyl-β-cyclodextrin on the solubility and stability of chlorambucil and melphalan in aqueous solution. Int. J. Pharm., 57: 63-72, 1989.

12. Pop, E., Loftsson, T. and Bodor, N. Solubilization and stabilization of a benzylpenicillin chemical delivery system by 2-hydroxypropyl-β-cyclodextrin. Pharm. Res., 8: 1044-1049, 1991.

13. Szejtli, J. Cyclodextrins in drug formulations: Part I. Pharm. Techn. Int., 3: 15-21, 1991.

14. Uekama, K., Fujinaga, T., Hirayama, F., Otagiri, M., Yamasaki, M., Seo, H., Hashimoto, T. and Tsuruoka, M. Improvement of the oral bioavailability of digitalis glycosides by cyclodextrin complexation. J. Pharm. Sci., 72: 1338-1341, 1983.

15. Bekers, O., Uijtendaal, E.V., Beijnen, J.H., Bult, A. and Underberg, W.J.M. Cyclodextrins in the pharmaceutical field. Drug Dev. Ind. Pharm., 17: 1503-1549, 1991.

16. Duchene, D., Vaution, C. and Glomot, F. Cyclodextrins, their value in pharmaceutical technology. Drug Dev. Ind. Pharm., 12: 2193-2215, 1986.

17. Hedges, A.R. (Ed.) Minutes of the Sixth International Symposium on Cyclodextrins, Editions de Sante, Paris, 1992.

18. Wheeler, L.A. The use of inclusion complexes of prostaglandins with cyclodextrins in the treatment of ocular hypertension. Eur. Pat., O 435 682 A3, 1991.

19. Usayapant, A., Karara, A.H. and Narurkar, M.M. Effect of 2-hydroxypropyl-β-cyclodextrin on the ocular absorption of dexamethasone and dexamethasone acetate. Pharm. Res., 8: 1495-1499, 1991.

20. Freedman, K.A., Klein, J.W. and Crosson, C.E. Beta-cyclodextrins enhance bioavailability of pilocarpine. Curr. Eye Res., 12: 641-647, 1993.

21. Rajewski, R.A., Stella, V.J., Tam, M., Waugh, W. and Thompson, D.O. Pharmaceutically useful anionic cyclodextrin derivatives. Pharm. Res., 9: S-227, 1992.

22. Rajewski, R.A. Development and evaluation of the usefulness and parenteral safety of modified cyclodextrins. Ph.D. Dissertation, University of Kansas, Lawrence, KS, USA, 1990.

23. Stella, V.J. and Rajewski, R.A. Derivatives of cyclodextrins exhibiting enhanced aqueous solubility and the use thereof. US Patent, 5,134,127, 1992.

24. Järvinen, T., Suhonen, P., Auriola, S., Vepsäläinen, J., Urtti, A. and Peura, P. Bispilocarpic acid monoesters as prodrugs of pilocarpine: I. Preparation and identification. Int. J. Pharm., 79: 233-242, 1992.

25. Järvinen, T., Suhonen, P., Auriola, S., Vepsäläinen, J., Urtti, A. and Peura, P. 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.

26. Gibaldi, M. and Perrier, D. In Pharmacokinetics. 2nd edition, Marcel Dekker Inc., New York, 1977, pp. 409-417.

27. Frijlink, H.W., Eissens, A.C., Schoonen, A.J.M. and Lerk, C.F. The effects of cyclodextrins on drug absorption II. In vivo observation. Int. J. Pharm., 64: 195-205, 1990.

28. Nakanishi, K., Masada, M., Nadai, T. and Miyajima, K. Effect of the interaction of drug-β-cyclodextrin complex with bile salts on the drug absorption from rat small intestinal lumen. Chem. Pharm. Bull., 37: 211-214, 1989.

29. Mitra, A.K. and Mikkelson, T.J. Ophthalmic solution buffer systems I. The effect of buffer concentration on the ocular absorption of pilocarpine. Int. J. Pharm., 10: 219-229, 1982.

30. Ahmed, I. and Patton, T. Effect of pH and buffer on the precorneal disposition and ocular penetration of pilocarpine in rabbits. Int. J. Pharm., 19: 215-227, 1984.

31. Mosher, G.L., Bundgaard, H., Falch, E., Larsen, C. and Mikkelson, T.J. Ocular bioavailability of pilocarpic acid mono- and diester prodrugs as assessed by miotic activity in the rabbit. Int. J. Pharm., 39: 113-120, 1987.

32. Schoenwald, R.D., Ward, R.L., DeSantis, L.M. and Roehrs, R.E. Influence of high-viscosity vehicles on miotic effect of pilocarpine. J. Pharm. Sci., 67: 1280-1283, 1978.

33. Chrai, S.S. and Robinson, J.R. Ocular evaluation of methylcellulose vehicle in albino rabbits. J. Pharm. Sci., 63: 1218-1223, 1974.

34. Schoenwald, R.D. and Ward, R.L. Relationship between steroid permeability across excised rabbit cornea and octanol-water partition coefficients. J. Pharm. Sci., 67: 786-788, 1978.

35. Mosher, G.L. and Mikkelson, T.J. Permeability of the n-alkyl p-aminobenzoate esters across the isolated corneal membrane of the rabbit. Int. J. Pharm., 2: 239-243, 1979.

36. Schoenwald, R.D. and Huang, H-S. Corneal behaviour of β-blocking agents I: physicochemical factors. J. Pharm. Sci., 72: 1266-1272, 1983.

37. Suhonen, P., Järvinen, T., Peura, P. and Urtti, A. Permeability of pilocarpic acid diesters across albino rabbit cornea in vitro. Int. J. Pharm., 74: 221-228, 1991.

38. Uekama, K. and Otagiri, M. Cyclodextrins in drug carrier systems. CRC Crit. Rev. Ther. Drug Carrier Syst., 3: 1-40, 1987.

39. Mitra, A.K. and Mikkelson, T.J. Mechanism of transcorneal permeation of pilocarpine. J. Pharm. Sci., 77: 771-775, 1988.

40. Choudhury, S. and Mitra, A. K. Kinetics of aspirin hydrolysis and stabilization in the presence of 2-hydroxypropyl-β-cyclodextrin. Pharm. Res., 10: 156-159, 1993.

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