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Preparation and Evaluation of Sustained Ophthalmic Gel of Enoxacin

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ABSTRACT The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of the drug may be overcome by the use of a gel system. The present work describes the formulation and evaluation of an ophthalmic delivery system containing an antibacterial agent, enoxacin, based on the concept of ophthalmic sustained gel, in which 2-hydroxypropyl-beta-cyclo-dextrin (HP-β-CD) was used as a penetration enhancer in combination with hydroxypropylmethyl-cellulose (Methocel F4M) which acted as a vehicle. The developed formulation was therapeutically efficacious, nonirritant, and provided sustained release of the drug over 8 h period in vitro and 7 h period in vivo. The developed system is a viable alternative to conventional eye drops.

KEYWORDS Sustained ophthalmic gel, Enoxacin, Hydroxypropyl-beta-cyclodextrin (HP-β-CD), Penetration enhancer, Hydroxypropylmethylcellulose (HPMC), Vehicle, Pharmacokinetics, Microdialysis

INTRODUCTION

Eye drops that are conventional ophthalmic delivery systems often result in poor bioavailability, and therapeutic response causes high tear turnover and dynamics causes rapid precorneal elimination of the drug (Mikkelson, 1984; Schoenwald, 1990). A high frequency of eye drop instillation is associated with patient noncompliance. Inclusion of excess drug in the formulation in an attempt to overcome bioavailability problems is potentially dangerous if the drug solution drained from the eye is systemically absorbed from the nasolacrimal duct (Middleton et al., 1990).

A significant increase in the precorneal residence time of drug and consequently, bioavailability, can be achieved along the following lines (Kaur & Smith, 2002):

1. To increase the transcorneal passage of drugs by incorporating absorption promoters/penetration enhancers into the drug formulations.
2. To optimize formulation vehicles for prolonged drug retention in the precorneal area and an increased contact time between the administered

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TABLE 1 Effect of HP-β-CD on Transcorneal Permeation of Enoxacin Under Study

HP-β-CD (w/v)	n	HL[a]% ± SD	P_{app} ($cm \cdot s^{-1} \times 10^6$ ± SD)	Relative permeability
GBR[b]	4	78.67 ± 1.37	1.795 ± 0.15	1
2%	4	78.35 ± 3.15	3.685 ± 0.41*	2.05
4%	3	78.91 ± 3.36	6.435 ± 1.23*	3.58
6%	3	80.92 ± 1.44	6.100 ± 0.47*	3.40
8%	3	81.80 ± 2.98	2.002 ± 0.26	1.12
12%	3	82.77 ± 2.48	1.995 ± 0.15	1.11

[a]Corneal hydration level.
[b]Gluathione bicarbonate ringer (GBR) buffer (no HP-β-CD).
*Significantly different ($P < 0.01$) from the control (GBR buffer, no HP-β-CD).

drug and the conjunctival and corneal epithelia by addition of water-soluble, natural, synthetic, or semi-synthetic viscolizers.

The objective of the present work was to develop a new sustained ophthalmic delivery system of enoxacin, a third-generation fluoroquinolone derivate used in external infections of the eye such as acute and subacute conjunctivitis, bacterial keratitis, and keratoconjunctivitis. The topical ophthalmic dose of enoxacin is 1–2 drops of a 0.3% solution in the affected eye(s) every 4 h or hourly in the case of severe infection. A combination of 2-hydroxypropyl-beta-cyclodextrin (HP-β-CD) and hydroxypropylmethylcellulose (HPMC) were investigated as penetration enhancer and vehicle, respectively, for the formulation of ophthalmic gels of enoxacin (0.3%, w/w), which would provide sustained release of the drug during treatment in ocular infections.

MATERIALS AND METHODS

Materials

Enoxacin was purchased from Wuhan Pharmaceutical Manufacture (Wuhan, China). 2-Hydroxypropyl-beta-cyclodextrin (HP-β-CD) was purchased from Shandong XinTai HengDa Chemical Co. Ltd., (Xintai, China). Lidocaine hydrochloride injection was purchased from Shanghai Hefeng Pharmaceutical Co. Ltd., (Shanghai, China). Ofloxacin ophthalmic solution was purchased from HuBei Qianjiang Pharmaceutical Manufacture (Qianjiang, China). Hydroxypropyl methyl cellulose (HPMC F4M) was kindly donated by Colorcon (UK). All the other chemicals were of analytical grade.

Animals

New Zealand white rabbits, weighing 2.5–3.0 kg, were offered by Animal Experimental Center of Shenyang Pharmaceutical University. The animals, housed in standard cages in a light-controlled room at 19±1°C and 50±5% relative humidity (RH), were given a standard pellet diet and water ad libitum. The animals were treated and used as indicated in the publication "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 92-93 revised 1985).

Preparation of Formulations

Selection of Penetration Enhancer

After the animals were killed by intravenous air through marginal vein of ear, freshly excised rabbit corneas were immediately mounted in a perfusion apparatus (Camber, 1985) which were thermostated at 35±1°C using a recirculating water bath (79HW-1, Zhejiang, China), where the corneal area available for diffusion was 0.70 cm^2. Preheated 35°C pH=6.85 glutathione bicarbonate Ringer (GBR) buffer (O'Brien & Edelhouse, 1977) was added to the epithelial (2.0 mL) and the endothelial (7.8 mL) compartment. To ensure oxygenation and agitation, an O_2–CO_2 (95:5) mixture was bubbled through each compartment at a rate of 3–4 bubbles/sec. After 10 min, the solution on the epithelial side was withdrawn and substituted with 2.0 mL of a 0.3% solution of drug in GBR buffer, with or without enhancer (the concentrations are indicated in Table 1). The drug concentration in the donor chamber was low to avoid possible drug effects on corneal integrity over prolonged incubation.

TABLE 2 Optimization of Vehicle

	Quality/*g*		
Formulation	A	B	C
Enoxacin	0.3	0.3	0.3
Methocel F4M	2	3	4
HP-β-CD	4	4	4
Acetic acid	2	2	2
Sodium hydroxide I.P.	8	8	8
Benzalkonium bromide	0.02	0.02	0.02
Purified water I.P.	100	100	100

At appropriate intervals, the entire contents of the receptor cells were withdrawn and the cells were refilled with fresh receiving solution equilibrated at (35±1)°C. Each experiment was continued for about 4.0 h, and performed at least in triple. The samples were filtered (0.45 μm) and analyzed by using a reversed high-performance liquid chromatography (HPLC) (Shimadzu LC-10ATvp) (ODS C_{18}, 5 μm, 200 mm × 4.6 mm; mobile phase: Acetonitrile:Methanol: H_2O=7:15:88(V:V:V), containing 0.05 mo·L^{-1} citromalic acid; flow rate 1.0 mL·min^{-1}; wavelength 266 nm; the retention time was 11–12 min).

The apparent permeation coefficients (P_{app}) in units of centimeters per second, defined by the expression (Fabrizio et al., 1996):

$$P_{app} = Q/(t \cdot C_0 \cdot A \cdot 60) \quad (1)$$

where A, the exposed corneal surface area (0.70 cm^2), and C_0, the initial permeant concentration, were calculated from the steady-state slopes of linear plots of the amount of drug in the receiving chamber (Q) versus time (t).

Determination of Corneal Hydration Levels (HL)

Wet corneal weight, W_a, was obtained after careful removal of the scleral ring; each corneal sample was then desiccated at 100°C for 6 h to give the corresponding dry corneal weight, W_b (Fabrizio et al., 1996).

The percent corneal hydration level (HL%), defined as $[1-(W_b/W_a)]\cdot 100$, was determined both on untreated corneas (removed no later than 30 min after the animals death) and on corneas recovered from permeation tests performed in the absence and in the presence of enhancers.

Optimization of Vehicle

Aqueous solutions of varying concentrations of HPMC F4M (A, B, C) were prepared as follows (Table 2). Methocel F4M was dissolved in 30 mL of purified water and allowed to hydrate overnight. The solution was stirred with overhead stirrer. Enoxacin was dissolved in acetic acid and pH was adjusted to 5.2 by sodium hydroxide. Benzalkonium bromide was then added and the solution was filtered through 0.22 μm cellulose acetate membrane filter. The drug solution was added to the above solution under constant stirring until uniform solution was obtained. Purified water was then added to make up the quality to 100 g.

The in vitro release of enoxacin from the gels was studied through 0.45 μm syringe membrane using a modified USP dissolution testing apparatus (27 mm i.d. and 5 mm in depth, diffusion surface 5.72 cm^2). The dissolution medium was artificial tear fluid freshly prepared (pH 7.4) (composition was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride $2H_2O$ 0.008 g, purified water q.s.1000 g) (Srivida et al., 2001). Syringe membrane, previously soaked overnight in the dissolution medium, was fixed between the containers. One gram of test gel was accurately pipetted into this assembly. The container was attached to the metallic drive shaft and immersed in 500 mL of dissolution medium maintained at (35±1)°C with rotating speed at 75 rpm. Samples, each of 10 mL volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The release of enoxacin was analyzed by HPLC.

Evaluation of Formulations

Ocular Irritation Studies

Ocular irritation studies were performed according to Draize technique (Draize et al., 1944) on six New

Zealand white rabbits, each weighing 2.5–3.0 kg. The samples, composed of 0.3% enoxacin, 4% HP-β-CD as enhancer, and 3% HPMC as vehicle, were instilled five times a day for a period of 7 days and the rabbits were observed periodically for redness, swelling, and watering of the eye. Evaluation was done as per Draize technique.

Pharmacokinetics Studies

Rabbits (n=4) were anesthetized with injection lidocaine hydrochloride injection. A custom-designed LM-10 microdialysis probe (Bioanalytical System, USA) was implanted into the anterior chamber of each eye as described (Rittenhouse et al., 1995). Probe inlet and outlet lines were tunneled beneath the conjunctiva, under the upper eyelid, and exited between the ears. The leads were protected with a latex glove pocket affixed to the top of the head. The probe was introduced as described previously (Rittenhouse et al., 1995), the anchor was sutured to the sclera with 7-0 Vicryl, and conjunctiva was sutured over the anchor. Exterior wound surfaces were treated with ofloxacin 0.3% ophthalmic solution. Animals were used for experimentation after >5 days recovery. Slit-lamp was taken after recovery to estimate fibrin formation and the condition of the eye prior to use of the rabbit in experiments.

Conscious rabbits (n=4) were placed in rabbit restrainers "home made" which permitted free movement of the head. Following a 1 h equilibration period with perfusion of saline through the probe, different concentration standard enoxacin saline solutions (0.109, 0.218, 0.436, 1.09, 2.18, 4.36, and 8.72 $\mu g \cdot mL^{-1}$) were perfused through the probe at a rate of 3 $\mu L \cdot min^{-1}$, and dialysate were collected for 15 min after 30 min of perfusion. A 20 μL aliquot of each fraction was analyzed by HPLC. In vivo recovery was defined as (Lonnroth et al., 1987):

$$R = (C_{in} - C_{out})/(C_m - C_{out})$$

where C_{in}=the concentration of standard solutions, C_{out}=the concentration of dialysate, and C_m=the concentration in aqueous humor. A linear equation was plotted by ($C_{in}-C_{out}$) vs. C_{out}, and the slope of the line gives the recovery (R).

After the disturbance of standard solutions was reduced to negligible by perfusion of saline through the probe, 40 μL of enoxacin gel as test or enoxacin ophthalmic solution as reference was placed in the lower cul-de-sac with a micropipette. In general, the rabbits closed their eyes without blinking after enoxacin administration. Immediately post-dose, 30 μL fractions of effluent were collected every 10 min for 1 h, then 60 μL collected every 20 min for 6 h. A 20 μL aliquot of each fraction was assessed by HPLC.

RESULTS AND DISCUSSION

Effect of HP-β-CD on the Corneal Permeability of Enoxacin

Linear permeation plots with correlation coefficients (r) in the range 0.986–0.999 were obtained in all cases, both in the absence and in the presence of HP-β-CDs. Table 1 listed the apparent permeation coefficients (P_{app}) and the corneal hydration levels (HL) determined for the permeant in GBR buffer alone, and in the presence of HP-β-CDs, at the sated concentrations.

As shown in Table 1, the permeability of HP-β-CD with different concentrations was in the order 12%≈8%<2%<6%<4%. A significantly lower transcorneal flux was measured for enoxacin under higher concentrations HP-β-CD (8% and 12%) with respect to lower concentrations (2%, 4%, and 6%).

These observations might be explained by two mechanisms. One is that CDs enhance drug permeability through a biological membrane such as the eye cornea and skin by disrupting the membrane either by permeating into the membrane or by extracting some lipophilic components, such as cholesterol and phospholipids, from the membrane (Duchêne & Wouessidjewe, 1996). The other is that CDs act mainly as carriers, which was reported by Másson (1998). In the case of aqueous eye drop solutions, CDs act as true carriers by keeping the hydrophobic drug molecules in solution and deliver them through the aqueous mucin layer to the surface of the ocular barrier (i.e., cornea or conjunctiva) where they partition into the barrier. According to this model, the delivery of drug through the aqueous mucin layer is diffusion controlled, but drug delivery through the membrane (e.g., cornea) is membrane controlled. These two mechanisms might exist simultaneously. At low CD concentrations, the permeability coefficient increases with increasing CD concentration. Under these conditions, the first

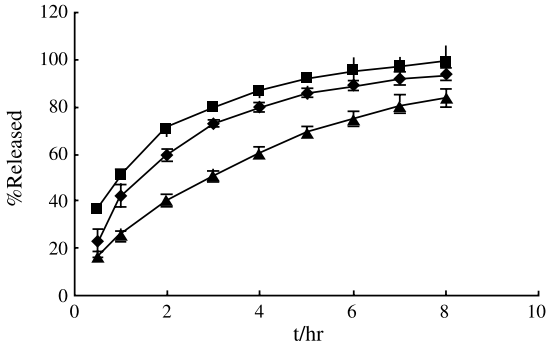

FIGURE 1 **Cumulative Amount of Enoxacin Released as a Function of Time from Various Enoxacin-Containing Gels. ■ = Enooxacin-Containing 2% HPMC Gel, ◆ = Enoxacin-Containing 3% HPMC Gel, and ▲ = Enoxacin-Containing 4% HPMC Gel (n = 6).**

mechanism might play the main role. At high CD concentrations, the second mechanism might play the main role. When excess CD is present, the permeability coefficient decreases with increasing CD concentration due to decreased drug activity in the donor phase (Higuchi, 1960).

HP-β-CD is a safe enhancer, without toxicity and irritancy, which is the most commonly applied CD in aqueous eye drop solutions (Loftsson & Stefánsson, 1997). The percent corneal hydration is a parameter frequently used to evaluate the damage of this tissue. According to Maurice and Riley (1970), the normal cornea has a hydration level of 76–80%. As indicated by Schoenwald and Huang (1983), an 83–92% hydration level, i.e., 3–7 percent units or moreover the "normal" value, denotes damage of the epithelium and/or endothelium. However, there are exceptions to this rule: according to Grass and Robinson (1988) high concentrations (>0.5%) of EDTA may produce substantial damage and expansion of the intercellular spaces of the corneal epithelium without influencing the normal hydration level.

In the present study, the average hydration level±SD of freshly exercised corneas was 77.25%± 1.37. As shown in Table 2, the hydration level was 78.67%±1.37 obtained from the corneas retrieved at the end of permeation runs in the absence of added HP-β-CDs. Higher hydration levels were observed in the presence of HP-β-CD with all concentrations. HL increase produced was less than 3 units, and not statistically different from the control.

Optimization of Vehicles

Figure 1 shows the cumulative amount of enoxacin release versus time profiles for drug-containing HPMC solutions. All HPMC solutions contained 0.3% (w/w) enoxacin. For the drug-containing 2% HPMC solution, about 37% of enoxacin was released after 0.5 h and 96% of enoxacin released after 6 h. In the case of drug-containing 3% HPMC solution, the drug released 23% and 90% to the medium after 0.5 h and 6 h, respectively. For 4% HPMC solution, lower drug release rates were observed. There was only 17% enoxacin released after 0.5 h, approximately 75% released after 6 h, and the release profile was still climbing hereafter. The results indicated that the 3% HPMC solution had better ability to retain drugs than 2% and 4% HPMC solutions. Furthermore, by plotting cumulative amount versus square foot of time curves for the various solutions (up to 80% of total drug released) linear relationships with correlation coefficients higher than 0.99 can be obtained. This observation was in accordance with the carbopol/hydroxypropyl-methylcellulose systems reported by Kumar et al. Kumar & Himmelstein, 1995. The linear relationships in conjunction with the slow dissolution rates suggest that the in vitro drug release from those polymer vehicles at physiological condition occurred primarily by diffusion.

Ocular Irritation Studies

The results of the ocular irritancy studies (Table 3) indicated that the formulation was nonirritant. Excellent ocular tolerance was noted. No ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae were visible.

Pharmacokinetics Studies

Figure 2 illustrates the linear regression between perfusate (C_{in}) and dialysate (C_{out}):

$$C_{in} - C_{out} = -0.3964C_{out} - 89.893 \ (r = 0.9985)$$

so the in vivo recovery (r) is 39.64%±6.17.

In vitro, there are a number of parameters effecting recovery which can be investigated. These parameters

TABLE 3 **Ocular Irritating Test (n = 6)**

Preparation	Average score
Blank of formulation	0
Formulation	0
Marked eye drop	0

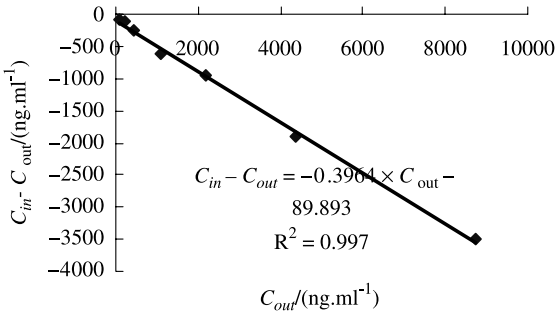

FIGURE 2 **In Vivo Recovery of Microdialysis Probe in Aqueous Humor (n=3).**

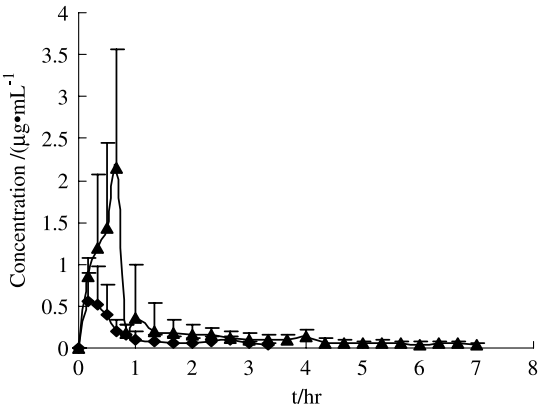

FIGURE 3 **Aqueous Humor Enoxacin Concentration-Time Profiles Following a 40 μL Topical Dose in Conscious Rabbits (n=4). □=Reference; ▲=Test.**

include perfusion flow-rate, temperature, perfusate composition, characteristics of the drug, characteristics of the semipermeable membrane, and the surface of the semipermeable membrane. All parameters that influence in vitro recovery will also influence in vivo recovery. However, in vivo, tissue characteristics will play an important role and may ultimately determine the recovery. In vivo recovery depends on diffusion in three regions: probe lumen, dialysis membrane, and the periprobe environment (Benveniste et al., 1991; Bungay et al., 1990, 1991). The first two regions can be characterized in vitro. Diffusion in probe lumen is limiting only with the use of very low flow rates. Diffusion through the dialysis membrane is limiting only when transport through the periprobe environment is rapid. Rapid diffusion through the periprobe environment occurs in most flowing system (like blood). In tissues, effective diffusion through the extracellular fluid determines the recovery of the microdialysis probes (Bungay et al., 1990, 1991).

In this study, in vivo recovery is almost as twice as reported (Fukuda et al., 1999; Othori et al., 1998; Sato et al., 1996): the recoveries were only 16%–20%. The difference exists in probe lumen and perfusion flow-rate.

The area under the aqueous humor conentration versus time (*AUC*) was estimated by the linear trapezoidal method with extrapolation to infinite time. Concentration at peak (C_{max}), time to peak (T_{max}), and terminal rate constant (K_e) were calculated with noncompartmental techniques (Gibaldi, 1998). Individual aqueous humor parameters for each eye were calculated. All parameters were reported as mean±SD.

Aqueous humor pharmacokinetic parameters were presented in Table 4. The *AUC* of test group is 3.8-fold versus the reference group ($P<0.05$), and the C_{max} of test group versus the control group is 3.9-fold ($P<0.1$). The T_{max} of test group is longer than that of reference group, and K_e of test group is lower than that of reference group.

As shown in Fig. 3, enoxacin could be still detected at 7 h after adminstration in the test group, otherwise, it could only be detected at 3.3 h after adminstration in the reference group. So the developed formulation has longer resident time in aqueous humor than conventional ophthalmic solutions.

CONCLUSION

A sustained ophthalmic gel was successfully prepared by using HP-β-CD as penetration enhancer and HPMC F4M as vehicle, with enoxacin as a model drug. The gel povided sustained release of the drug over an 8 h period in vitro. The formulation was therapectically efficacious. Ocular irritancy test indicated the formulation is nonirritant. Pharmacokinetic

TABLE 4 **Pharmacokinetics Parameters of Enoxacin in Aqueous Humor After Topical Administration in Conscious Rabbit (n=4)**

Group	*AUC* ($\mu g \cdot mL^{-1} \cdot h \cdot \mu g^{-1}$)	C_{max} ($\mu g \cdot mL^{-1} \cdot \mu g^{-1}$)	T_{max} (h)	K_e
Reference	0.414±0.21	0.689±0.89	0.25±0.10	0.548±0.50
Test	1.614±0.90**	2.726±1.79*	0.33±0.24	0.270±0.14

*$P<0.10$.
**$P<0.05$.

study indicated the formulation has a higher bio-availability and longer residence time in aqueous humor than conventional ophthalmic solutions. The developed formulation is a viable alternative to conventional eye drops due to its ability to enhance bio-availability through its longer precorneal residence time and ability to sustain release of the drug. Also important is the ease of administration afforded and decreased frequency of administration resulting in better patient compliance.

REFERENCES

Benveniste, H., Hansen, A. J., & Ottosen, N. S. (1991). Determination of brain interstitial concentrations by microdialysis. *Journal of Neurochemistry*, *57*, 1741–1750.

Bungay, P. M., Morrison, P. F., & Dedrick, R. L. (1990). Steady-state theory for quantitative microdialysis of solutes and water in vivo and in vitro. *Life Sciences*, *46*, 105–119.

Bungay, P. F., Hsiao, J. K., Ball, B. A., Mefford, I. N., & Dedrick, R. L. (1991). Quantitative microdialysis: analysis of transient and application to pharmacokinetics in brain. *Journal of Neurochemistry*, *57*, 103–119.

Camber, O. (1985). An in vitro model for determination of drug permeability through the cornea. *Acta Pharmaceutica Suecica*, *78*, 815–819.

Draize, J. H., Woodard, G., & Calvery, H. O. (1944). Methods for the study of irritation and toxicity of substances. *Journal of Pharmacology and Experimental Therapeutics*, *82*, 377–390.

Duchêne, D., & Wouessidjewe, D. (1996). Pharmaceutical and medical applications of cyclodextrins. In: S. Dumitriu (Ed.), *Polysaccharides in Medical Applications*. New York: Marcel Dekker, 575–602.

Fabrizio, S. M., Chetoni, P., Cerbai, R., Mazzanti, G., & Braghiroli, L. (1996). Evaluation of ocular permeation enhancers: in vitro effects on corneal transport of four β-blockers, and in vitro/in vivo toxic activity. *International Journal of Pharmaceutics*, *142*, 103–113.

Fukuda, M., Mikitani, M., & Ueda, T. (1999). Application of microdialysis on analysis of pharmacokinetics in domestic rabbit aqueous humor. *Journal of Japanese Ophthalmologic Society*, *99*, 400–405.

Gibaldi, M. (1998). Regulating angiogenesis: a new therapeuric strategy. *Journal of Clinical Pharmacology*, *38*, 898–903.

Grass, G. M., & Robinsion, J. R. (1984). Relationship of chemical structure to corneal penetration and influence of low-viscosity solution on ocular bioavailability. *Journal of Pharmaceutical Sciences*, *73*, 1021–1027.

Higuchi, T. (1960). Physical chemical analysis of percutaneous absorption process from creams and ointments. *Journal of the Society of Cosmetic Chemists*, *11*, 85–97.

Kaur, I. P., & Smith, R. (2002). Penetration enhancers and ocular bioadhesives: two new avenues for ophthalmic drug delivery. *Drug Development and Industrial Pharmacy*, *28*(4), 353–369.

Kumar, S., & Himmelstein, K. J. (1995). Modification of in situ gelling behavior of carbopol solutions by hydroxypropyl methylcellulose. *Journal of Pharmaceutical Sciences*, *84*(3), 344–348.

Loftsson, I., & Stefánsson, E. (1997). Effect of cyclodextrins on topical drug delivery to the eye. *Drug Development and Industrial Pharmacy*, *23*, 473–481.

Lonnroth, P., Jansson, P. A., & Smith, U. (1987). A microdialysis method allowing characterization of intercellular water space in humans. *American Journal of Physiology*, *253*, 228–231.

Masson, M., Loftsson, T., Masson, G., & Stefansson, E. (1999). Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing. *Journal of Controlled Release*, *59*, 107–118.

Maurice, D. M., & Riley, M. V. (1970). Ocular pharmacokinetics. In: C. N. Gragmore (Ed.), *Biochemistry of the Eye*. London: Academic Press, 6–16.

Middleton, D. L., Leung, S. S., & Robison, J. R. (1990). Lenarts, V., R. Gurny (Eds.), *Bioadhensive Drug Delivery System*. Boca Raton, FL: CRC Press, 179–202.

Mikkelson, T. J. (1984). Ophthalmic drug delivery. *Pharmaceutical Technology*, 90–98.

O'Brien, W. J., & Edelhouse, H. F. (1977). The corneal penetration of trifluorothymidine, adenine arabinoside, and idoxuridine: a comparative study. *Investigative Ophthalmology & Visual Science*, *16*(12), 1093–1103.

Othori, R., Sato, H., & Fukuda, S. (1998). Pharmacokinetics of topical beta-adrenergic antagonists in rabbit aqueous humor evaluated with the microdialysis method. *Experimental Eye Research*, *66*, 487–494.

Rittenhouse, K. D., Jr., Periffer, R. L., & Pollack, G. M. (1995). Evaluation of microdialysis. *Journal of Japanese Ophthalmologic Society*, *7*, 513–519.

Sato, H., Fukuda, S., & Inatomi, M. (1996). Pharmacokinetics of norfloxacin and lomefloxacin in domestic rabbit aqueous humor analyzed by microdialysis. *Journal of Japanese Ophthalmologic Society*, *100*, 513–519.

Schoenwald, R. D. (1990). Ocular drug delivery: pharmacokinetic considerations. *Clinical Pharmacy*, *18*(4), 255–269.

Schoenwald, R. D., & Huang, H. S. (1983). Corneal penetration behavior of β-blocking agents I: Physico chemical factors. *Journal of Pharmaceutical Sciences*, *72*, 1266–1272.

Srivida, B., Cardoza, R. M., & Amin, P. D. (2001). Sustained ophthalmic delivery of ofloxacin from a pH-triggered in situ gelling system. *Journal of Controlled Release*, *73*, 205–211.