International Journal of Pharmaceutics, 53 (1989) 219–225
Elsevier

IJP 01804

Solution viscosity effects on the ocular disposition of cromolyn sodium in the albino rabbit

Vincent Hon-Kin Li [1,2] and Joseph R. Robinson [1]

[1] *School of Pharmacy, University of Wisconsin, Madison, WI 53706 (U.S.A.) and* [2] *Allergan Pharmaceuticals, Irvine, CA 92715 (U.S.A.)*

(Received 22 November 1988)
(Modified version received 11 January 1989)
(Accepted 11 January 1989)

Key words: Ocular disposition; Cromolyn sodium; Viscosity; Rabbit; Radiotracer; Conjunctiva; Polymer; Opticrom

Summary

The influence of viscosity on the ocular disposition of cromolyn sodium has been studied in albino rabbits using a radiotracer technique. A commercial cromolyn sodium solution, Opticrom, was used as a control; and test solution viscosities, in the range of 580–3200 cP, were adjusted with hydroxypropyl methylcellulose. Concentrations of cromolyn sodium were determined in tears, cornea, aqueous humor, iris–ciliary body, and conjunctiva, in that order. The higher iris–ciliary body and aqueous humor drug levels from viscous solutions, as compared to that of Opticrom, despite identical corneal drug levels, suggest an alternate intraocular penetration route besides the cornea. In general, the influence of viscosity on ocular disposition of cromolyn sodium is modest. In comparison to the Opticrom solution, viscous solutions promoted higher levels of cromolyn sodium in the tears, iris–ciliary body, aqueous humor (except the 3200 cP solution), and conjunctiva but not in the cornea. Mixing of viscous solutions with tears in the cul-de-sac is poor. Slow elimination of cromolyn sodium from conjunctiva, cornea, and iris–ciliary body suggests binding to these tissues.

Introduction

Topical application of drugs to the eyes is significantly impeded by the efficient removal processes that exist at the site of drug delivery. Thus, typically less than 3% of an applied dose penetrates to the aqueous humor (Benson, 1974). One traditional way to alleviate this problem is to increase viscosity of the instilled solution by incorporation of water-soluble polymers such as polyvinyl alcohol, methylcellulose and hydroxymethylcellulose. The assumption is made that drug absorption will be improved by increasing contact time with the absorptive surfaces. Contact time can be prolonged by increasing viscosity of the vehicle in which the drug is formulated (Patton and Robinson, 1975; Chrai and Robinson, 1974; Saettone et al., 1980). However, low viscosity solutions, i.e., less than 100 cP, are only slightly more effective than non-viscous aqueous solutions (Patton and Robinson, 1975; Grass and Robinson, 1984) in improving ocular bioavailability, using the albino rabbit as the test animal.

When a viscous polymer solution is instilled into the cul-de-sac, its viscosity will be reduced by several processes. Firstly, the viscous solution is

Correspondence: J.R. Robinson, School of Pharmacy, University of Wisconsin, Madison, WI 53706, U.S.A.

diluted by resident tears and subsequently by incoming tears. Secondly, the viscous solution will undergo shear thinning during blinking, thereby increasing contact area and facilitating mixing. These two dynamic processes, together with the steep viscosity-polymer concentration relationship, modify the influence of viscosity on contact time, which, though prolonged, is still relatively brief.

On the positive side, increasing viscosity does prolong contact time and should, therefore, promote absorption. On the negative side, it can slow diffusion of drug in solution and, more importantly, can create mixing problems of the drug solution with tears. In addition, the polymer may absorb onto absorptive surfaces, creating a barrier for drug penetration. Furthermore, increasing discomfort due to increasing viscosity may induce lacrimation. Finally, the albino rabbit is reported to be less sensitive to viscous solutions than humans due to their decreased blink rate (Saettone et al., 1982). All of these factors may affect the expected improvement in drug bioavailability.

Cromolyn sodium, also known as sodium cromoglycate, is used prophylactically in the treatment of allergic and vernal keratoconjunctivitis. The mechanism of action is not well understood, but is thought to act by inhibiting degranulation of mast cells with subsequent release of histamine and other mediators of the allergic response (Garland et al., 1976). Recent studies by Lee et al. (1983) have demonstrated that 5% PVA (about 50 cP) solution could promote higher cromolyn levels in various ocular tissues. The purpose of the present study was to evaluate the influence of medium viscosity solutions, i.e., 580–3200 cP, on the ocular disposition of cromolyn sodium in the albino rabbit.

Materials and Methods

Materials

Opticrom[1] cromolyn sodium and ^{14}C-labeled cromolyn sodium, spec. act. 11.8 μCi/mg and of greater than 99% purity, were obtained from Fisons [2]. Hydroxypropyl methylcellulose (Methocel 4000) was obtained commercially [3]. All other chemicals were either reagent or analytical grade and were used as received.

Male albino rabbits [4], weighing 2.5–3.0 kg, were used throughout the studies. They were fed a regular diet with no restriction on food and water consumption.

Methods

Solution preparation. Different viscosity cromolyn sodium solutions were prepared by mixing an appropriate amount of cromolyn sodium solution and 4% hydroxypropyl methylcellulose (HPMC) solution. The final formulation contained 2% (w/w) cromolyn sodium and the final viscosities of the viscous solutions were 580 cP, 1025 cP, and 3200 cP. The pH of the solutions were 7.0 ± 0.1. Viscous solutions for animal studies were freshly prepared by spiking the drug solution with an appropriate amount of ^{14}C-labeled cromolyn sodium before mixing with the 4% HPMC solution. A 25-mg test solution gave about 1,000,000 dpm. Apparent viscosities of the test solutions were determined at 25°C using a Brookfield viscometer [5]. Rheograms of the viscous solutions were generated using a Haake viscometer [6] equipped with a coaxial cylinder system consisting of an MV cup and an MV I rotor. Opticrom solution was spiked with [^{14}C] labeled cromolyn sodium prior to each experiment.

Administration of the viscous solutions. During the experiments, all rabbits were restrained in an upright posture. Individual doses, about 25 mg, of the 3200 cP cromolyn sodium solutions were weighed on an analytical balance and carefully transferred to a microspatula and then placed inside the center of the lower cul-de-sac with care being taken not to touch the corneal surface. For the less viscous solutions, i.e., 1025 cP and 580 cP,

[1] Registered trademark of Fisons plc, Loughborough, U.K.

[2] Fisons plc, Pharmaceutical Division, Loughborough, U.K.

[3] Dow Chemical Company, Midland, MI, U.S.A.

[4] Klubertanz, Edgerton, WI, U.S.A.

[5] Model LVT, Brookfield Engineering Laboratories, Stoughton, MA, U.S.A.

[6] Model Rotovisco RV 12, Haake, Saddle Brook, NJ, U.S.A.

instillation of the dose was by means of a pipetman [7]. It was found by weight measurements that approximately 10% variation in dose can be expected with this method of delivery. During dosing, the lower eyelid was pulled away from the globe to form a pocket, while the upper eyelid was slightly raised. Immediately after dosing, both eyelids were released and no further mechanical manipulation was performed.

Concentration–time profiles of cromolyn sodium in tears, aqueous humor, cornea, iris–ciliary body, and conjunctiva

Tear film. Approximately 10 s prior to sacrifice of the test animal with an overdose of sodium pentobarbital solution, a 1-μl tear sample was withdrawn from the center of the lower marginal tear strip using a disposable, 1 μl glass pipet. Pipets containing tear samples were transferred to minivials [8] containing 0.5 μl of doubly-distilled water. 4.5 ml scintillation cocktail [8] was added an hour later. The minivials were vortexed for about 15 s to ensure good mixing of the water and scintillation cocktail.

Anterior ocular tissues. Following sacrifice of the test animal, corneal and conjunctival surfaces were rinsed with normal saline and carefully blotted dry to remove residual solution. Anterior ocular tissues – aqueous humor, cornea, iris–ciliary body, and conjunctiva – were obtained in that order. 150 μl of aqueous humor samples were transferred to scintillation vials containing 15 ml of scintillation cocktail. Each of the tissue samples were digested at 55 °C for 18 h in 1 ml of a tissue solubilizer [9] contained in a glass scintillation vial and then decolorized by the addition of 100 μl of hydrogen peroxide. To this solution was added 15 ml of Aquasol.

Both the tissue samples and the tear samples were stored in the dark for 24 h prior to counting in a liquid scintillation spectrometer [10]. The presence of micropipets in the scintillation cocktails did not alter the counting efficacy or affect the result in any way. After correcting for background and quenching, the data in disintegrations per min were converted to mg drug through the use of standards, assuming no drug metabolisms occurred in various tissues as was the case for systemic administration (Ashton et al., 1978).

Statistical analysis

A one-way ANOVA was used in computation of test of significance for more than two groups, while comparison between two groups, was by means of an unpaired *t*-test.

Results and Discussion

Rheograms of viscous cromolyn sodium solutions at 25 °C and 34 °C are displayed in Fig. 1. Regardless of shear rate, the 3200 cP solution gave the highest apparent viscosity. Pseudoplastic behavior typical of HMPC solutions was observed at both temperatures. Viscosities of the solutions decrease as temperature increases from 25 °C to 34 °C.

Table 1 shows the time course of cromolyn levels in the tear film, and Table 2 lists the AUC of the concentration–time profiles of cromolyn sodium in the tear film and the various ocular tissues. Regardless of vehicle viscosity, the concentration of cromolyn sodium is highest in the tears, followed by the conjunctiva, cornea, iris–ciliary body, and aqueous humor, in that order. A one-way ANOVA test shows no difference ($P < 0.05$) in tear cromolyn sodium levels among the Opticrom solution and the three viscous cromolyn sodium solutions at 5 min. However, significantly higher drug levels ($p < 0.05$) were observed in the case of viscous solutions between 10 and 90 min. The higher tear drug levels from viscous solutions were probably due to their slower removal from the precorneal area. Among the viscous solutions, tear drug levels were highest for the 580 cP solution at the earlier times. Such a seemingly anomalous result appears to be due to better mixing between the 580 cP solution and tears, its easier flow into the 1 μl micropipet, and

[7] Model P200, Rainin Instruments, Woburn, MA, U.S.A.
[8] RPI Research Products International, MT. Prospect, IL, U.S.A.
[9] Aquasol, New England Nuclear, Boston, MA, U.S.A.
[10] Protosol, New England Nuclear, Boston, MA, U.S.A.
[11] Model 2002, Packard Instruments, Down Grove, IL, U.S.A.

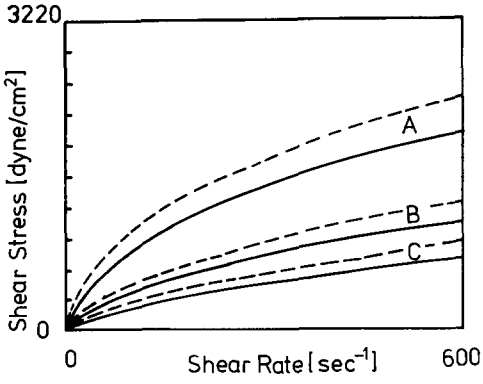

Fig. 1. Rheograms of 2% cromolyn sodium-hydroxypropyl methylcellulose solutions at 25 °C and 34 °C. (— — —), 25 °C; (———), 34 °C; A, 3200 cP; B, 1025 cP; C, 580 cP.

the fact that most drug is associated with the unmixed viscous solution at earlier times.

Drug concentrations attained in various ocular tissues are dependent on the tear film drug concentration, which serves as the driving force for drug diffusion across the absorptive surfaces. Thus, one would expect larger AUC (Table 2) of the concentration–time profiles for the cornea (Table 3) from the viscous solutions as compared to Opticrom. However, the results indicate that there was no difference ($p < 0.05$) between the AUC of the Opticrom solution and the viscous solutions. A possible explanation for such inconsistency may be due to the fact that the observed tear film concentrations may not represent the true free

TABLE 2

Area under curve (AUC) of the tear levels and tissue levels of cromolyn sodium as a function of viscosity

Drug	Opticrom	580 cP	1025 cP	3200 cP
Tear *				
AUC **	1333	4055	3225	2910
AUC ***	–	4175	3387	3012
Cornea				
AUC **	10.4	10.9	11.9	9.8
AUC ***	–	18.0	17.7	15.3
Aqueous humor				
AUC **	0.45	0.96	1.02	0.51
AUC ***	–	1.50	1.48	0.87
Iris–ciliary body				
AUC **	2.41	5.31	5.83	6.83
AUC ***	–	10.33	11.3	11.75
Conjunctiva				
AUC **	18.8	30.8	53.4	29.8
AUC ***	–	65.9	92.8	56.5

For tear and aqueous humor, units of AUC: μg/ml·h; and for cornea, iris–ciliary body and conjunctiva, units of AUC: μg/g·h.

* Assume an initial concentration of 15,625 μg/ml based on a 7 μl resident tear volume. ** AUC (0–180 min). *** AUC (0–360 min).

TABLE 1

Cromolyn sodium concentration – time profiles in tear film

Time (min)	Opticrom	3200 cP	1025 cP	580 cP
5	3909 (2432)	2746 (1207)	4051 (2415)	5341 (3014)
10	1148 (850)	2658 (1243)	2849 (782)	5817 (2360)
15	990 (670)	–	–	–
20	436 (195)	1841 (947)	2645 (1480)	4134 (2240)
30	500 (407)	1284 (916)	1833 (901)	1544 (1112)
45	–	1363 (1087)	1493 (640)	1880 (1384)
60	380 (125)	725 (438)	583 (243)	614 (816)
90	44 (36)	855 (651)	468 (459)	745 (721)
120	76 (92)	88 (94)	230 (136)	173 (122)
180	44 (51)	35 (24)	119 (99)	40 (32)
240	–	38 (47)	44 (45)	48 (33)
300	–	32 (34)	35 (24)	34 (21)
360	–	30 (18)	48 (28)	36 (16)

Concentrations in μg/ml. Values are mean (S.D.) of 8–10 determinations.

TABLE 3

Cromolyn sodium concentration – time profiles in cornea

Time (min)	Opticrom	3200 cP	1025 cP	580 cP
5	5.18(1.76)	4.41(1.67)	4.86(2.15)	6.49(3.84)
10	4.87(2.45)	5.61(1.97)	8.79(4.13)	4.56(2.93)
15	5.93(1.94)	–	–	–
20	4.77(1.40)	2.66(1.30)	6.35(2.31)	9.11(6.14)
30	4.22(1.97)	3.82(2.04)	6.33(2.71)	3.53(1.43)
45	–	3.02(1.43)	3.64(1.21)	4.12(1.88)
60	4.77(2.07)	4.20(1.69)	3.86(2.26)	3.76(1.30)
90	3.28(0.90)	2.84(1.06)	3.11(1.32)	3.70(2.50)
120	2.87(1.14)	3.47(0.64)	3.34(1.69)	3.16(1.37)
180	2.36(0.64)	2.31(0.82)	3.30(1.73)	1.74(0.42)
240	–	1.87(1.15)	1.33(1.20)	1.63(0.51)
300	–	1.42(0.89)	1.51(0.67)	1.84(1.03)
360	–	2.12(1.04)	2.50(1.34)	1.45(0.48)

Concentrations in μg/ml. Valves are mean (S.D.) of 8–10 determinations.

TABLE 4

Cromolyn sodium concentration – time profiles in aqueous humor

Time (min)	Opticrom	3200 cP	1025 cP	580 cP
5	0.054(0.017)	0.061(0.020)	0.120(0.045)	0.080(0.061)
10	0.063(0.025)	0.101(0.058)	0.199(0.098)	0.117(0.053)
15	0.089(0.035)	–	–	–
20	0.123(0.058)	0.181(0.140)	0.152(0.067)	0.255(0.155)
30	0.129(0.039)	0.144(0.108)	0.482(0.260)	0.203(0.088)
45	–	0.133(0.057)	0.354(0.180)	0.234(0.144)
60	0.203(0.087)	0.153(0.056)	0.299(0.176)	0.467(0.388)
90	0.172(0.093)	0.174(0.059)	0.397(0.270)	0.349(0.244)
120	0.162(0.055)	0.262(0.142)	0.423(0.240)	0.416(0.131)
180	0.152(0.058)	0.123(0.037)	0.286(0.134)	0.265(0.099)
240	–	0.131(0.082)	0.111(0.044)	0.178(0.106)
300	–	0.116(0.112)	0.145(0.090)	0.158(0.113)
360	–	0.088(0.061)	0.133(0.082)	0.157(0.058)

Concentrations in μg/ml. Values are mean (S.D.) of 8–10 determinations.

TABLE 5

Cromolyn sodium concentration – time profiles in conjunctiva

Time (min)	Opticrom	3200 cP	1025 cP	580 cP
5	6.65(3.28) *	17.16 (7.67)	37.69(19.4)	16.97 (8.86)
10	8.22(3.09)	23.38(13.6)	45.34(22.9)	15.27 (4.98)
15	11.66(6.05)	–	–	–
20	8.59(4.95)	7.48 (3.86)	45.61(26.1)	24.89(16.8)
30	8.78(3.58)	10.12 (4.72)	21.92(16.6)	9.52 (5.02)
45	–	11.03 (5.61)	25.27(11.6)	10.89 (4.33)
60	5.42(3.20)	9.99 (7.08)	12.81(11.9)	9.70 (6.61)
90	4.58(1.78)	10.40 (6.37)	13.40 (9.81)	9.79 (4.17)
120	3.45(3.51)	8.49 (4.34)	11.23(10.4)	7.68 (3.62)
180	2.53(1.46)	8.00 (5.42)	12.23 (6.18)	7.67 (5.04)
240	–	9.34 (5.18)	9.91 (6.02)	14.97(15.1)
300	–	9.08 (7.37)	14.87 (8.02)	13.41 (9.81)
360	–	8.68 (6.32)	17.12(10.5)	5.84 (4.36)

Concentrations in μg/ml. Values are mean (S.D.) of 8–10 determinations.

drug concentrations available for drug transport across the cornea. This is likely considering that mixing between the viscous solutions and tears is usually poor and drug has to diffuse out of the polymer solution before it can reach the absorptive surface.

Assuming drug in the aqueous humor (Table 4) comes entirely from the cornea, and on the basis of cornea levels alone, one would expect that the aqueous humor concentration–time profiles of the Opticrom solution and the viscous solutions should be more or less identical. Results indicate that there is no statistical difference ($p < 0.05$) between the AUC of aqueous humor (Table 2) of the Opticrom solution as compared to that of the 3200 cP but about one-half that of the 1025 cP or 580 cP solution. The reason for such disparity is unclear and inconsistent with the cornea data. However, it is possible that drug in aqueous humor comes not only from cornea but also from iris–ciliary body, which derives its drug probably from the conjunctiva via the sclera. Several recent studies have demonstrated this alternate pathway of intraocular penetration (Bito and Baroody, 1982; Maurice and Polgar, 1977; Ahmed and Patton, 1984). The higher conjunctival and iris–ciliary body drug levels (Tables 5 and 6) from viscous solutions also seem to support such a contention.

The low aqueous humor drug levels suggest slow movement of cromolyn sodium through the cornea, probably because of its size and charge, and/or binding to the cornea. Slow movement of the drug through the cornea into the aqueous humor coupled with unfavorable partitioning favor back-diffusion of drug into the tears, which partly accounts

TABLE 6

Cromolyn sodium concentration – time profiles in iris – ciliary body

Time (min)	Opticrom	3200 cP	1025 cP	580 cP
5	0.72(0.22) *	1.26(0.73)	2.37(1.67)	0.83(2.01)
10	1.12(0.26)	1.12(0.46)	2.37(1.38)	3.89(2.28)
15	1.05(0.25)	–	–	–
20	1.24(0.32)	0.94(0.42)	4.40(3.13)	2.87(2.29)
30	0.79(0.33)	2.76(0.93)	1.66(1.49)	1.20(0.83)
45	–	1.59(1.74)	2.81(1.70)	2.78(2.11)
60	0.82(0.22)	2.94(0.93)	1.47(1.33)	1.79(0.68)
90	0.84(0.27)	2.04(0.72)	1.75(1.19)	1.32(0.78)
120	0.74(0.21)	3.06(0.78)	1.65(0.98)	1.59(0.41)
180	0.74(0.20)	2.09(1.09)	2.19(1.53)	1.20(0.48)
240	–	1.57(0.86)	1.98(1.70)	1.94(1.02)
300	–	1.29(0.81)	1.61(0.96)	2.04(0.94)
360	–	1.94(0.70)	1.47(0.31)	1.35(0.42)

Concentrations in μg/ml. Values are mean (S.D.) of 8–10 determinations.

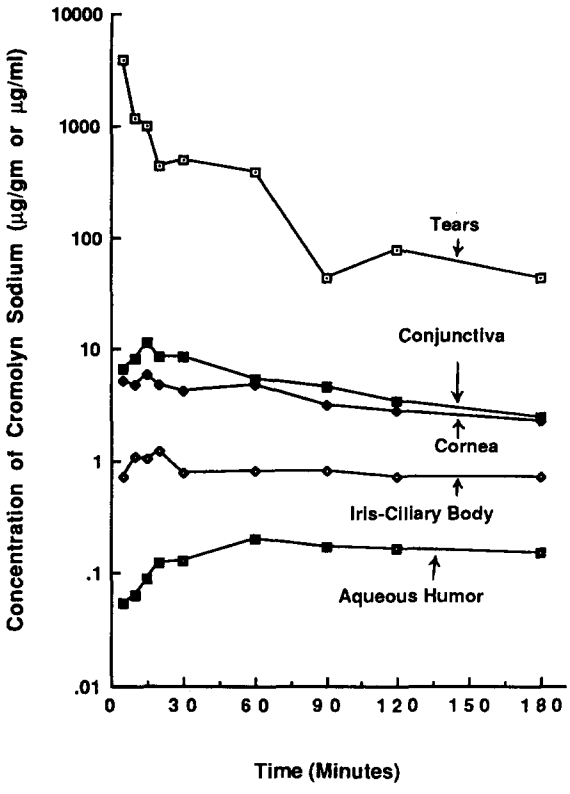

Fig. 2. Concentration–time profiles of cromolyn sodium in the tears, cornea, aqueous humor, iris-ciliary body, and conjunctiva following topical instillation of Opticrom solution. It is expressed as μg/ml for tears and aqueous humor, and μg/g for cornea, iris–ciliary body, and conjunctiva. The rank order of drug levels in various ocular tissues was observed to be the same for viscous solutions. Between 8–10 eyes were used for each time point. Error bars are omitted for the sake of clarity.

for removal of drug away from the cornea especially at later times postinstillation.

The AUC of the concentration–time profiles of the iris-ciliary body (Table 2) of the 3 viscous solutions are identical ($P < P0.05$) and are about twice that of the Opticrom solution. The consistently higher drug levels in the iris–ciliary body compared to that in the aqueous humor at all times postinstillation suggests binding and/or involvement of the alternate pathway.

Table 5 shows the concentration–time profiles of cromolyn sodium from the Opticrom and viscous solutions. Viscous polymer solutions were able to promote higher drug levels in the conjunctiva than the Opticrom solution. This is a fortunate occurrence assuming that mast cells in the conjunctiva are the primary target. At earlier times, the 1025 cP solution provided the highest drug concentrations, but from 60 min onward, there is no significant difference ($P < 0.05$) among the 3 viscous solutions. The slow elimination of cromolyn sodium from the conjunctiva, a higher vascularized tissue, suggests binding.

In summary, increasing viscosity did promote higher drug levels in the conjunctiva, iris–ciliary body, and aqueous humor but not in the cornea. The indifference of the corneal cromolyn sodium levels in response to solution viscosity is unclear. The higher drug levels in aqeous humor, iris–ciliary body, and conjunctiva from the viscous solutions compared to that of the Opticrom, despite identical corneal drug levels, suggests the possibility of an alternative intraocular route of penetration. Sustained drug levels in the iris–ciliary body and slow drug elimination from the conjunctiva are likely to be due to binding. Recent studies by Lee et al. (1983) found similar results. Although increasing viscosity promoted higher cromolyn sodium levels in the conjunctiva, the primary target tissue, the improvement appears to reach a maximum around 1000 cP. From the results of this study, it appears undesirable to increase viscosity of the instilled solution to more than 1000 cP in the case of cromolyn sodium and possibly for most ophthalmic drugs (Grass and Robinson, 1984) because of the likelihood of promoting other undesirable effects associated with viscous solutions.

References

Ahmed, I. and Patton, T.F., Importance of the noncorneal absorption route in topical ophthalmic drug delivery. Presented at the *37th National Meeting of Academy of Pharmaceutical Sciences, Oct. 28–Nov. 1, 1984.*

Ashton, M.J., Clark, B., Jones, K.M., Moss, G.F., Neale, M.G. and Ritchie, J.T., The absorption, metabolism and excretion of disodium cromoglycate in nine animal species. *Toxicol. Appl. Pharmacol.*, 26 (1973) 319.

Benson, H., Permeability of the cornea to topically applied drugs. *Arch. Ophthalmol.*, 91 (1974) 313.

Bito, L.Z. and Baroody, R.A., The penetration of exogenous prostaglandin and arachidonic acid into, and their distribu-

tion within, the mammalian eye. *Curr. Eye Res.*, 1 (1982) 659.

Chrai, S.S. and Robinson, J.R., Ocular evaluation of methylcellulose vehicle in albino rabbits. *J. Pharm. Sci.*, 63 (1974) 1218.

Garland, L.G. and Foreman, J.C., Cromoglycate and other antiallergic drugs: a possible mechanism of action. *Br. Med. J.*, 1 (1976) 820.

Grass, G.M. and Robinson, J.R., Relationship of chemical structure to corneal penetration and influence of low-viscosity solution on ocular bioavailability. *J. Pharm. Sci.*, 73 (1984) 1021.

Lee, V.H.L., Stratford, R.E. Jr., Morimoto, K.W. and Swarbrick, J., Disposition of topically applied sodium cromoglycate in the albino rabbit eye. *J. Pharm. Pharmacol.*, 35 (1983) 445.

Maurice, D.M. and Polgar, J., Diffusion across the sclera. *Exp. Eye Res.*, 25 (1977) 577.

Patton, T.F. and Robinson, J.R., Ocular evaluation of polyvinyl alcohol. *J. Pharm. Sci.*, 64 (1975) 1312.

Saettone, M.F., Giannacini, B., Savigini, P. and Writh, A., The effect of different ophthalmic vehicles on the activity of tropicamide in man. *J. Pharm. Pharmacol.*, 32 (1980) 519.

Saettone, M.F., Giannacini, B., Barattini, F., and Tellini, N., The validity of rabbits on ophthalmic vehicles: a comparison of four vehicles containing tropicamide in humans and rabbits. *Pharm. Acta Helv.*, 57 (1982) 47.