Vehicle effects in ophthalmic bioavailability: an evaluation of polymeric inserts containing pilocarpine

M. F. SAETTONE*, B. GIANNACCINI, P. CHETONI, G. GALLI** AND E. CHIELLINI†

*Institute of Pharmaceutical Chemistry, **Institute of Industrial Organic Chemistry and †Institute of General Chemistry (Faculty of Engineering), University of Pisa, 56100 Pisa, Italy*

A series of polymeric ophthalmic inserts containing pilocarpine were formulated with four different types of polyvinyl alcohol, PVA, and two types of hydroxypropylcellulose. Pilocarpine was present as the nitrate, or as the salt with polyacrylic acid, PAA. In-vivo miosis vs time experiments on albino rabbits, showed that all inserts increased significantly the bioavailability of pilocarpine, with respect to a standard solution of pilocarpine nitrate. Two PVA inserts, containing the PAA-salt of pilocarpine, were particularly effective. The preparations were also submitted to in-vitro release tests and to differential scanning calorimetry, to ascertain the release mechanism, and to verify, via the thermal behaviour, possible interactions between drug and polymers. The chemical and physicochemical factors, most likely to influence the ophthalmic bioavailability of pilocarpine from the present preparations, are briefly reviewed.

Poor penetration of topically applied drugs into the anterior segment of the eye is a well-known disadvantage in ophthalmic therapy. Liquid, and, to a lesser extent, semisolid medications are rapidly diluted and removed from the absorption area by different concurring mechanisms such as reflex tearing, blinking and tear turnover. Thus, frequent administration of eyedrops is necessary to maintain an adequate level of certain drugs, e.g. anti-glaucoma agents, in the aqueous humour. The addition of suitable polymers to liquid collyria may result in a bioavailability increase by prolonging the corneal contact time (Olejnik et al 1982; Saettone et al 1982), however, no real sustaining effect can be obtained by this method. The commonly available ophthalmic ointments, although better retained than collyria, do not efficiently release all types of drugs and they are ill-tolerated by many patients. These factors have stimulated the search for alternative ophthalmic dosage systems, aimed at ensuring a prolonged time of residence of the medication in the eye and, possibly, a controlled release, while avoiding a pulse entry of the drug. A variety of solid delivery systems, responding totally or partially to the above requisites, have been developed in recent years. Medicated contact lenses, polymeric monolithic inserts and complex, zero-order diffusional systems may be mentioned as examples (Richardson 1975).

The present study had the purpose of evaluating, on a physicochemical and a biological basis, a series of commercially available polymers as possible substrates for the preparation of soluble, monolithic inserts containing pilocarpine. Forms of this type have been used (cf. the glycerinated gelatin 'lamellae' of the 1948 B.P.), and were lately revived in the USSR by Maichuk (1975), who investigated inserts based on synthetic polymeric materials. The latter dosage systems, while not ensuring a zero-order release, have been reported to favour the penetration and to prolong the action of the medicinal agent, besides ensuring its stability (Maichuk 1975). The present investigation was considered as a preliminary step to the formulation of new polymeric materials, specifically tailored as ophthalmic drug carriers and insert formers.

MATERIALS AND METHODS

Polymers
The following commercially available polymers were used as received: polyvinyl alcohol, PVA (Polyviol, Wacker Chemie GmbH); hydroxypropylcellulose, HPC (Klucel, Hercules Inc.); polyacrylic acid, PAA (Carbopol 940, Goodrich Chemical Co.). Their essential physicochemical properties are summarized in Table 1.

Pilocarpine
Pilocarpine nitrate (m.p. 176–178 °C, E. Merck, Darmstadt) was used as received. Pilocarpine base was obtained from the nitrate by extraction with chloroform of the alkalinized (NH_4OH, pH 8–9)

Table 1. Physicochemical characteristics of the tested polymers.

Polymer	Sample	Average mol. wt ($\times 10^{-3}$)	$\overline{HD}\%$ [a]	T_m (°C) [b]
PVA	A	25	86–89	185
	B	72	99–100	225
	C	90	97–99	215
	D	72	97–99	215
HPC	E	500	—	210
	F	100	—	200
PAA	G	>1000	—	nd [c]

[a] Degree of hydrolysis referred to the parent poly(vinyl acetate). [b] Melting temperature, determined by DSC. [c] No DSC endothermic peaks detected up to 270 °C.

solution. A 2·0% w/v solution of pilocarpine nitrate in pH 5·5 isotonic phosphate buffer (hereafter referred to as Psol), and a viscous gel containing 1·54% w/v pilocarpine base and 0·77% w/v PAA (hereafter referred to as Pgel) were used as reference standards for the biological tests. A sample of pilocarpine-PAA salt for the DSC study was prepared as follows: to a PAA dispersion (2·75 g, 27·5 mequiv) in 50 ml of anhydrous benzene, was added a solution of pilocarpine base (5·75 g, 27·5 mequiv) in 50 ml of the same solvent. The mixture was stirred at room temperature (20 °C) for 1 h, the solvent was evaporated under reduced pressure and the white powdery residue was washed with anhydrous ether and was dried under vacuum. The final product contained 67% pilocarpine base (nitrogen analysis).

Preparation of inserts
Transparent, flexible films were obtained by slow evaporation, at 50 °C, of aqueous 5·0 w/v solutions of the polymers containing the appropriate amount of pilocarpine nitrate. The films (0·4–0·5 mm thickness) were cut in the form of small disks, each containing 1·0 ± 0·05 mg drug. PVA films containing pilocarpine-PAA salt were prepared by adding to the 5·0% w/v polymer solution the appropriate amount of pilocarpine base, and PAA in small excess over the equivalent amount. The resulting viscous gels were liquified by ultrasonic treatment, then were evaporated as described before. The final inserts contained in this case 0·768 mg pilocarpine base, i.e. an amount corresponding to that contained in 1·0 mg pilocarpine nitrate. All inserts were routinely analysed for pilocarpine by hplc after thorough extraction with methanol, according to Dunn et al (1981). The procedure allowed a practically quantitative recovery of the drug; in no case conversion to isopilocarpine was observed. Hplc inserts containing

the pilocarpine-PAA salt could not be prepared on account of incompatibility between the two polymeric materials.

Biological studies
Miosis-time data on rabbits (male albino, 2–2·5 kg) were obtained on unanaesthetized preconditioned animals, by placing 50 μl of Psol, or Pgel, or one insert into the lower conjunctival sac of one eye of the animals, the other eye serving as control. The measurements were made at intervals, under standardized lighting conditions, by estimating to the nearest 0.1 mm the horizontal diameter of the pupil with a micrometer held always by the same operator at the same distance from the animal's eye. Each vehicle or insert was tested on groups of at least 10 different animals; in no case was irritation, or expulsion of the insert observed.

Release in-vitro
In-vitro release tests were made on 200 mg samples of polymer films, by determining pilocarpine release to a stirred aqueous medium (10·0 ml of pH 6·98 phosphate buffer) at 30 °C. Solution samples (2·0 ml) were withdrawn at appropriate intervals, and were replaced with an equal amount of fresh buffer. Pilocarpine was analysed spectrophotometrically by the ferric hydroxamate method of Gibbs & Tuckerman (1970).

DSC studies
Differential scanning calorimetry (DSC) tests were carried out on a Perkin-Elmer DSC-2 apparatus. Polymer samples and inserts (8–10 mg) and equivalent amounts of pilocarpine salts (ca 1·0 mg) were analysed at heating-cooling rates of 20 °C min^{-1} under dry nitrogen flow. Indium standards were used for temperature calibration and enthalpy change evaluation.

RESULTS AND DISCUSSION
Typical miosis-time data for some representative preparations under study are illustrated in Fig. 1, while the main activity parameters of all preparations are summarized in Table 2. Administration to test animals of 50 μl of Psol (2·0% w/v pilocarpine nitrate), corresponding to 1·0 mg of drug, produced a miosis of relatively short duration (ca 2 h), declining rapidly after reaching the peak effect. Conversely, the PVA or HPC inserts containing the same amount of pilocarpine nitrate, while not producing significantly higher peak miosis intensities, showed a
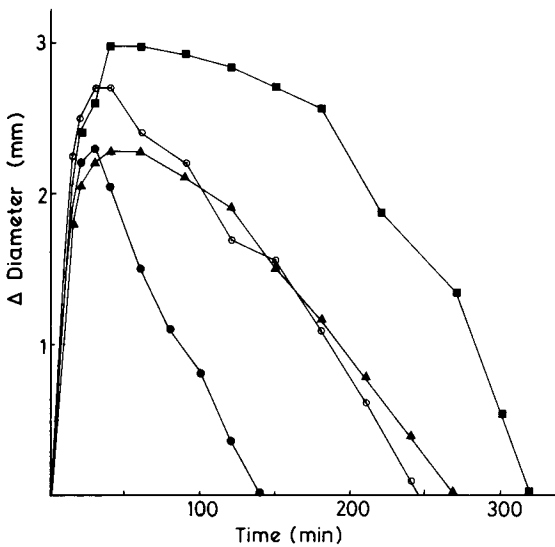
FIG. 1. Mean change in pupillary diameter *versus* time for some representative preparations. Key ●, solution Psol; ○, gel Pgel; ▲, PVA-C insert with pilocarpine nitrate; ■, PVA-C insert with pilocarpine PAA-salt.

slightly increased peak time (40 min), and a significantly prolonged effect (average value, 4·13 h). All except one (PVA-A) of the pilocarpine nitrate inserts produced an activity plateau of relatively short duration (range, 20–50 min). The areas under the miosis-time curves (AUC) of the preparations are of particular interest: their values should reflect the aqueous humour concentration of the drug, thus being indicative of the bioavailability of pilocarpine from each vehicle. It clearly appears from the data in Table 2 that administration of 1·0 mg pilocarpine nitrate in the solid inserts (PVA-A, B, C, D and HPC-E and F) produced a statistically significant ($P < 0.05$) bioavailability increase over the aqueous

solution (1·73 to 2·54 times). The AUC values for the PVA-A, B, C, D and HPC-E inserts containing nitrate were not statistically different from each other. The HPC-F insert showed a slightly, but significantly ($P < 0.05$) greater AUC value with respect to PVA-A and B. The fact that some inserts, shortly after being placed into the conjunctival sac, became viscous liquids (PVA-A and HPC-F) or a gel-like semisolid (HPC-E), while all others maintained their integrity and disk shape until the miotic effect had disappeared, apparently had no relevance to the specific activity.

The highest AUC values and longest times of activity were obtained with the PVA inserts containing the pilocarpine-PAA salt. Within this series, the best results were offered by PVA-C and D, which produced a 4-times bioavailability increase over the aqueous solution, and an average 5-h duration with a 110 min plateau. The other two inserts containing the PAA-salt (PVA-A and B) showed significantly lower AUC and duration values. In all cases, however, the inserts containing the PAA salt showed greater activity parameters with respect to the corresponding inserts containing the nitrate. Out of the four inserts containing the PAA-salt, only one (PVA-A) assumed a gel-like structure in the conjunctival sac, while all others preserved their integrity.

Interestingly, the Pgel showed significantly higher activity parameters (I_{max}, duration, AUC) with respect to PVA inserts A and B containing pilocarpine nitrate, while it had practically the same activity as the other inserts containing this salt.

In-vitro release experiments for all inserts which maintained their integrity during the in-vivo tests (PVA-B, C and D, with nitrate and PAA-salt) showed a linear relationship between fraction of

Table 2. Summary of the activity data in rabbits of pilocarpine (nitrate or PAA-salt) in different preparations.

Vehicle	I_{max}	TP	D	P	AUC	Relative AUC
Solution Psol	2·30 ± 0·32	30	138 ± 5	—	82·4 ± 14·6	1·00
Gel Pgel	2·70 ± 0·20	30	290 ± 10	—	195·7 ± 24·3	2·37
Insert PVA-A	2·44 ± 0·28	40	200 ± 20	—	142·9 ± 25·7	1·73
,, PVA-A/PAA	2·90 ± 0·21	40	258 ± 12	—	225·8 ± 23·6	2·74
,, PVA-B	1·92 ± 0·19	40	248 ± 11	50	144·5 ± 23·9	1·75
,, PVA-B/PAA	2·76 ± 0·22	40	287 ± 12	90	256·8 ± 33·4	3·12
,, PVA-C	2·28 ± 0·33	40	266 ± 18	40	186·5 ± 40·2	2·26
,, PVA-C/PAA	2·98 ± 0·20	40	320 ± 11	110	337·5 ± 43·8	4·09
,, PVA-D	2·28 ± 0·28	40	274 ± 18	50	186·6 ± 34·3	2·26
,, PVA-D/PAA	2·87 ± 0·26	40	318 ± 11	110	355·1 ± 42·7	4·31
,, HPC-E	2·58 ± 0·41	40	290 ± 21	20	209·6 ± 45·6	2·54
,, HPC-F	2·88 ± 0·45	40	213 ± 12	50	201·3 ± 31·1	2·45

I_{max} = peak height, mm ± 95% C.L. TP = time to peak, min. D = duration of miotic activity, min ± 95% C.L. P = activity plateau, min. AUC = area under the activity vs. time curve, cm² ± 95% C.L.

drug released and square root of time, up to 70–80% release. This indicated diffusion as the most important mechanism contributing to release from the polymeric inserts under the in-vitro conditions (Flynn et al 1974); a similar mechanism should be presumably operative also in-vivo. The main in-vitro release data are summarized in Table 3.

Table 3. In-vitro release data

Insert/Drug salt	Release 'rate' $(F/\sqrt{t}) \cdot 10^2$	Drug released after 24 h (%)
PVA-B/nitrate	1·39	100
PVA-B/PAA salt	0·95	88
PVA-C/nitrate	2·0	100
PVA-C/PAA salt	1.54	92
PVA-D/nitrate	2·08	100
PVA-D/PAA salt	1·43	89

F = fraction released; t = time in seconds.

The release 'rates' (corresponding to the slopes of the initial portions of the F, fraction of drug released, vs square root of time plots) for the inserts containing the PAA-salt were in all cases lower with respect to the rates calculated for the nitrate-containing inserts, thus indicating a slower release. Furthermore, the PAA-inserts retained ca 10% bound drug after a 24-h desorption, whereas the nitrate-containing inserts had completely released their drug content. In-vitro release, however, was comparatively fast in all cases, 60–80% of the drug being released to the aqueous phase within 30 min.

Possible interactions between the polymeric matrixes and the drug were investigated by submitting raw polymer materials, drug-free and drug-loaded inserts, and the two pilocarpine salts to DSC tests. The heating curves of all polymer samples were characterized by a rather sharp, intense melting peak (cf. e.g. curve a in Fig. 2). The temperature values corresponding to the endotherms maxima are reported in Table 1. The inserts showed temperature and enthalpy changes similar to those of the corresponding parent homopolymers, thus indicating that no DSC-appreciable chemical or physicochemical modifications had occurred during the preparation of the films. Pilocarpine nitrate showed a melting endotherm at 176 °C, followed by an overwhelming exotherm centred at 190 °C, and by a further exothermic transition at 220 °C (Fig. 2, curve b). In a previous report (Saettone et al 1983) the first exotherm had been tentatively attributed to isomerization of pilocarpine to isopilocarpine. However, this hypothesis should be discarded on the basis of
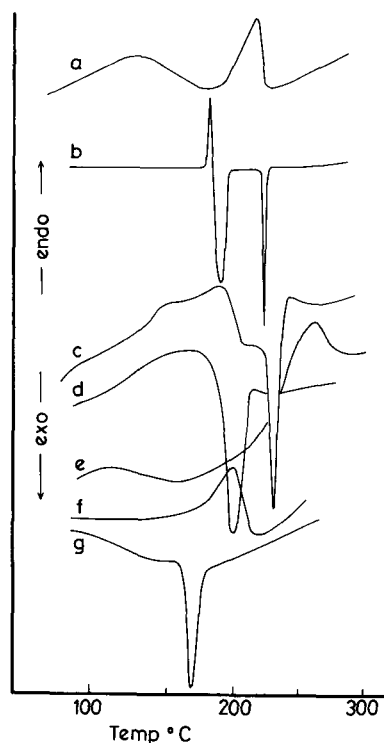

FIG. 2. Differential Scanning Calorimetry (DSC) thermograms of some representative materials. Key: (a) PVA-C film; (b) pilocarpine nitrate; (c) PVA-C insert with pilocarpine nitrate; (d) PVA-A insert with pilocarpine nitrate; (e) pilocarpine PAA-salt; (f, g) PVA-C insert with pilocarpine PAA-salt, heating and cooling, respectively.

further evidence. Indeed, pilocarpine base and the hydrochloride, which are known to isomerize on heating at about 200 °C (Petit & Polonowski 1897; Hill & Barcza 1966) did not show any exothermic transition, possibly attributable to isomerization, up to 250 °C, while isopilocarpine nitrate showed the same exothermic peaks (190 and 220 °C) observed with pilocarpine nitrate. Thus, since the pilocarpine-isopilocarpine transition does not involve thermal phenomena, the observed exotherms should be put into relation with the presence of the nitrate ion. Both pilocarpine and isopilocarpine nitrates apparently decompose on melting, with a strong evolution of gas and discolouration. A preliminary examination of the decomposition mixture from pilocarpine nitrate revealed the presence, besides unidentified tarry products, of isopilocarpine. The decomposition reaction is probably complex.

The inserts containing pilocarpine nitrate exhibited a complex thermal behaviour, with exothermic transitions due to nitrate decomposition, along with the expected endothermic transitions attributable to

the melting of the polymer. Interestingly, the occurrence of the exotherms appeared dependent on structural parameters of the PVA matrix, such as molecular weight and degree of hydrolysis. In the thermograms of inserts prepared with high-molecular weight and almost completely hydrolysed PVA (e.g., PVA-C), a strong, single exotherm was present at 230 °C, analogous to that observed with pilocarpine nitrate (Fig. 2, curve c). On the contrary, inserts prepared with low-molecular weight and incompletely hydrolysed PVA (PVA-A), showed a broad exotherm at 190 °C (Fig. 2, curve d). These exotherms probably correspond to the nitrate decomposition. That they may be due to degradation products (or isopilocarpine) interacting with the polymer cannot be excluded.

On cooling, in all cases broad exothermic peaks with two distinct minima were observed in the range 160–110 °C. In the event of derivative-polymer interactions occurring at high temperature, the cooling data have probably no great relevance. The sample having been taken past the transition temperatures the cooling curves should involve the products of this transition, and not the original pilocarpine-polymer mixture.

In any case, the heating data seem to indicate that host polymer and drug behave independently of each other, even though non-bonded or weak hydrogen bond interactions may play a role in determining the conformational assembling of pilocarpine nitrate in the matrix. A completely different behaviour was shown by the inserts containing pilocarpine-PAA salt. While the salt (Fig. 2, curve e) showed a broad melting transition at 260 °C, which was absent in the thermogram of polyacrylic acid G, all inserts, with the exception of PVA-B, had crystalline melting temperatures lower than those of the corresponding drug-free films, whereas no peaks attributable to the melting of the drug salt could be observed. On cooling, polymer crystallization occurred within a narrow temperature interval, with a relatively small degree of supercooling (cf. curves f and g, Fig. 2). These thermal features point to the existence of strong chemical interactions among matrix, polymeric anion and drug, which in turn may concur in establishing a tight binding of pilocarpine, and consequently a more restrained mobility and release.

CONCLUSIONS

The following points may be indicated as the main outcome of this study. (a) The polymers tested were selected from 20 commercial products. While other, yet untested, polymers may display better properties, the PVA types indicated as C and D appeared as the most promising insert formers, both on a biological and on a technological basis. (b) Salification of pilocarpine base with polyacrylic acid exerted a profound influence on drug bioavailability, as shown e.g. by the Pgel, which was as active as the nitrate-containing inserts. This influence was significantly enhanced when the PAA-salt was administered as a dispersion in a PVA insert. The former effect had been partially anticipated in the literature. Some authors (Loucas & Haddad 1972, 1976; Schoenwald & Roehrs 1981) have indicated an activity enhancement resulting from ophthalmic administration of polyanionic-polymer salts of pilocarpine. The further bioavailability enhancement resulting from administration of the salt dispersed in an appropriate PVA matrix appears interesting, and worthy of further investigation. (c) Thermal analysis has proved a sensitive and potentially useful technique for the assessment of basic drug-matrix interactions in prolonged release.

The reported data highlight the relevance to ophthalmic availability both (i) of the type of polymer forming the insert matrix, and (ii) of the diffusive properties of the drug base, that may be regulated by the polyanion structure. Concerning (i), a previous study (Saettone et al 1982) in which several iso-viscous liquid vehicles prepared with different polymers were compared, had indicated the superiority of PVA over other viscosity-inducing agents, thus ruling out an effect of solution bulk viscosity itself in ophthalmic bioavailability. In the case of the present inserts, partial dissolution of the matrix should result in formation of a polymer film, which, in the case of PVA, is probably endowed with an optimal miscibility with the tear film, stability or surface viscosity. Pilocarpine would diffuse from this precorneal film, held to a constant concentration by the cul-de-sac reservoir, to the corneal epithelium. As for (ii), evidence of interactions between the drug and the PAA-G polymer, presumably resulting in a decreased in-vivo rate of release of pilocarpine from the insert to the precorneal film, has been gathered both from in-vitro release studies and from DSC data. A slower delivery rate of the drug from the PVA/PAA-salt matrix, resulting practically in a sustained effect, is in all evidence due to the restraint imposed by the polyanionic macromolecular structure on the diffusing material.

Chimica Fine e Secondaria). The authors wish to thank FARMIGEA S.p.A., Pisa, for kindly providing experimental animals and testing facilities.

REFERENCES

Dunn, L. D., Scott, B. S., Dorsey, E. D. (1981) J. Pharm. Sci. 70: 446–449

Flynn, G. L., Yalkowski, S. H., Roseman, T. J. (1974) Ibid. 63: 479–509

Gibbs, I. S., Tuckerman, M. M. (1970) Ibid. 59: 395–396

Hill, R. K., Barcza, S. (1966) Tetrahedron 22: 2889–2893

Loucas, S. P., Haddad, H. M. (1972) J. Pharm. Sci. 61: 985–986

Loucas, S. P., Haddad, H. M. (1976) Metabol. Ophthalmol. 1: 27–34

Maichuk, Y. F. (1975) Invest. Ophthalmol. 14: 87–90

Olejnik, O., Stevens, J., Wilson, C. G., Hardy, J. G. (1982) J. Pharm. Pharmacol. 34: 28P

Petit, A., Polonowski, M. (1897) Chem. Zentr. 68: 361–362

Richardson, K. T. (1975) Arch. Ophthalmol. 93: 74–86

Saettone, M. F., Giannaccini, B., Teneggi, A., Savigni, P., Tellini, N. (1982) J. Pharm. Pharmacol. 34: 464–466

Saettone, M. F., Giannaccini, B., Chetoni, P., Galli, G., Chiellini, E. (1983) in: Chiellini, E., Giusti, P. (eds) Polymers in Medicine, Biomedical & Pharmacological Applications. Plenum Press, New York, pp 187–199

Schoenwald, R. D., Roehrs, R. E. (1981) U.S. Pat. No 4,271,143 to Alcon Laboratories, Inc.