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Evaluation of buffer systems in ophthalmic product development

Imran Ahmed [1] and Bhaskar Chaudhuri *

School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209 (U.S.A)

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Summary

Pilocarpine nitrate solutions, pH 4.0, containing equimolar (0.0667 M) concentrations of acetic acid/sodium acetate, mono-/dibasic sodium phosphate and citric acid/sodium citrate buffers were employed examine the effect of buffer capacity and buffering agents on ocular drug absorption in rabbits. Two new buffer parameters, termed average buffer capacity and residual buffer capacity, were introduced to elucidate the importance of buffer functionality and ionization values on the in vivo time course of lacrimal fluid pH. Buffer effect on the precorneal disposition of pilocarpine and inulin were also quantitated. Buffered solutions caused more induced lacrimation than an unbuffered solution at equivalent pH. Acetate buffer was found to cause excessive lacrimation, and may be inherently irritating to the eye. The utility of assessing the time course of lacrimal fluid pH and drug concentration to predict relative pilocarpine absorption efficiency was demonstrated. Pilocarpine was found to be less ocularly available from a phosphate-buffered solution than from the apparently more strongly buffered acetate formulation. This was attributed to phosphate exerting a resistance to pH re-equilibration near the pK_a of pilocarpine due to its high residual buffer capacity. However, phosphate may still be regarded as the buffer of choice for pilocarpine since it appears to cause less irritation to the eye. The procedures described in this report may be adapted for ophthalmic preformulation studies to screen buffer systems or other formulation excipients.

Introduction

Selection of a buffer system which ensures optimum dosage form performance is an integral part of ophthalmic product research and development. It is often found that the buffering requirements for drug stability and solubility are inconsistent with the requirements for optimum drug performance in the eye. An example of this is the case for pilocarpine. Pilocarpine is usually buffered at an acidic pH to overcome stability constraints (Chung et al., 1970), although this strategy has been shown to reduce its ocular bioavailability (Mitra and Mikkelson, 1982).

The study described in this report was undertaken with 3 specific objectives: (1) to examine, using pilocarpine as a model ionizable compound, how physicochemical buffer properties and the choice of specific buffering agents affect the time course of pH and drug disposition in the precorneal area; (2) to correlate precorneal drug disposition with drug bioavailability in the eye; and (3) to establish the practical utility of tear film pH and precorneal drug concentration measurement

* *Present address*: College of Pharmacy, Xavier University of Louisiana, New Orleans, LA 70125, U.S.A.
Correspondence: I. Ahmed. *Present address*: Pfizer Central Research, Eastern Point Road, Groton, CT 06340, U.S.A.

in routine ophthalmic preformulation studies to screen buffer systems.

Materials and Methods

Materials

Inulin (mol. wt. 5000) and pilocarpine nitrate were obtained from Sigma Chemicals, St. Louis, MO. All other chemicals used were analytical reagent grade. Radiolabeled [^{3}H]pilocarpine and the liquid scintillation fluor Aquasol II were purchased from New England Nuclear, Boston, MA. Radiolabeled [^{3}H]inulin was obtained from Amersham, Arlington Hts., IL. In vivo tear pH was determined using pH-sensitive paper (ColorpHast, E. Merck, Darmstadt, F.R.G.). One microliter microcapillary pipets (Microcaps, Drummond Scientific, Bromall, PA) were used to collect tear samples. White New Zealand rabbits, weighing 2.2–2.4 kg at the time of use, were purchased from Delta Biologicals, Tuscon, AZ. No restrictions were placed on food or water intake by the animals prior to experimentation.

Solution preparation

The composition of the ophthalmic test solutions used in the study are given in Table 1. Each solution contained 0.2% inulin and 1.0% pilocarpine nitrate in order to keep the drug effect uniform throughout the study. The solutions were rendered isotonic by adding sodium chloride. The buffered solutions were equimolar and differed only in the type of buffer used. The unbuffered solution was prepared in water and served as a control. All the solutions were prepared by dissolving the required amount of inulin, pilocarpine nitrate and sodium chloride in appropriate volumes of the vehicles and the pH was adjusted to 4.0 with HCl or NaOH. All in vitro studies and those studies requiring tear pH measurement and miosis measurement were performed using unlabeled drug solutions. For in vivo measurement of inulin and pilocarpine levels in the tears, radiolabeled solutions were prepared by adding to the "cold" solutions a tracer quantity of either [^{3}H]inulin or [^{3}H]-pilocarpine nitrate as to yield a total radioactivity of 100,000 cpm per dose in the instilled solution. In these studies inulin served as a poorly absorbed marker to quantitate the extent of pH-induced lacrimation, precorneal drug loss due to drainage and tear turn-over and, to reflect the precorneal disposition of the buffer species.

TABLE 1

Composition of the ophthalmic test solutions

Vehicle	water	water	water	water
Buffer	Acetate	Phosphate	Citrate	–
NaCl (% w/v)	0.42	0.21	0.22	0.67
Inulin (% w/v)	0.20	0.20	0.20	0.20
Pilocarpine nitrate (% w/v)	1.0	1.0	1.0	1.0
pH	4.0	4.0	4.0	4.0
β_{calc} (M)	0.023	0.002	0.042	0.0005
β_{expt} (M)	0.017	0.002	0.038	0.0007
	(0.0011) [1]	(0.0002)	(0.0015)	(0.00005)
β_{avg} (M×pH)	0.048	0.054	0.109	0.015

[1] The standard error of 4 determinations.

Buffer capacity measurement

Theoretical buffer capacity, β_{calc}, for the test solutions were calculated using the Van Slyke buffer equation (Van Slyke, 1922). Practical buffer capacity, β_{expt} of the test solutions and the lacrimal fluid was determined by titration. Titration was accomplished by adding 5–25 μl aliquots of a standardized 1 N NaOH solution to 10 ml of the test solutions or 1.5 ml of lacrimal fluid. The resultant solution pH was recorded after each addition until the physiological tear pH or ~ 7.4 was achieved. The molar buffer capacity at various pH values, ranging from 4.0 to 7.4, were calculated from the slope of the titration curve (Martin et al., 1983).

Collection of tear samples

Three 1.5 ml portions of pooled tear samples were collected from 8 conscious rabbits after blocking the nasolacrimal duct (puncta) with a polyethylene plug. The collected tear samples were expressed under mineral oil, acidified with 5 μl of 1 N HCl, and subsequently titrated with NaOH to determine the buffer capacity.

Lacrimal fluid pH–time profiles

The 4 test solutions were administered in single 25 μl doses to 6 conscious rabbits. The in vivo tear pH was determined at 0.5, 1, 3, 5, 7 and 10 min post-instillation by placing a pH indicator paper in contact with the tear film inside the lower cul-de-sac. The pH paper was held in contact with the tears for 3 s and the pH value was determined by comparing the resultant color against a color chart provided by the manufacturer. The accuracy of the pH paper readings was assessed to be within ± 0.2 pH units (Ahmed and Patton, 1984).

Precorneal drug loss

The 4 test solutions were administered in single 25 μl doses to 6 conscious rabbits. The study was repeated with solutions containing radiolabeled insulin and radiolabeled pilocarpine in order to assess the precorneal disposition for each compound in the formulation. One μl tear samples were collected at 1, 3, 5, 7 and 10 min using microcapillary tubes. After sampling, the tubes were placed in a liquid scintillation vial prefilled with 5 ml of Aquasol II. Standards were prepared by taking a series of 1 μl samples of the dosing solution. The standards and samples were analyzed for total radioactivity using a previously described LSC procedure (Ahmed and Patton, 1984).

Miosis–time profiles

Miosis profiles were obtained in rabbits to assess the extent of pharmacological activity and ocular availability of pilocarpine from the various test formulations. Miosis measurements were made using the procedure described previously by Mitra and Mikkelson (1982). The 4 test solutions were administered topically as single 25 μl doses to 6 conscious rabbits in a cross-over study. Experiments were conducted in a quiet, uniformly lit room. The animals were acclimated to dosing and handling for several days prior to data collection. Measurements were repeated by two individuals and compared to establish consistency. The accuracy of the measurements were ± 0.1 mm. In each animal, 3 baseline pretreatment pupillary diameter measurements were taken. After instillation, pupillary diameter measurements were taken at 10, 20, 30, 45 and 60 min. Average changes in pupil diameter (ΔPD) were reported as a function of time after instillation.

Results and Discussion

Acetate, phosphate and citrate buffers at concentrations approximating those used in this study are commonly found in commercial ophthalmic solutions of pilocarpine. The acceptable pH range for ophthalmic products is 6.0–8.0 (Mullins, 1980), but the eye is able to tolerate products outside this range if lightly buffered (Gourley and Makoid, 1986). The buffers selected for this study differed in terms of their functionality and dissociation constants. The acetate (pK_a 4.76) and phosphate (pK_2 7.20) buffers undergo only one dissociation between pH 4 and 7.4, while citrate buffer undergoes two dissociations (pK_2 4.76; pK_3 6.40) within this pH re-equilibration range. In addition, the first proton dissociation constant for citric acid (pK_1 3.13) also contributes to the formulation buffer capacity at pH 4.0.

The buffer capacities in the test formulations were evaluated both in vitro and in vivo. In general, the in vivo pH–time course in the tears for the formulations were consistent with the in vitro predictions. At the pH of formulation, the citrate-buffered solution had the highest buffer capacity, followed by the acetate and phosphate-buffered formulations. As expected, the unbuffered formulation had the lowest buffer capacity, although it did manifest a slight resistance to pH change due to the contribution to the buffer capacity by pilocarpine. In all cases, the experimentally determined buffer capacities were in agreement with the calculated values (Table 1).

The titration results revealed that the tears exerted a substantial buffering effect at acidic pH, but not at near-physiological pH (Fig. 1). This is indicative of the physiological function of the tears to protect the eye against an external pH insult. At pH 4.0, the molar buffer capacity of the lacrimal fluid was substantially higher than the buffer capacities of the unbuffered and phosphate-buffered formulations. Consequently, only a moderate depression in the tear pH resulted after instillation of these formulations. On the contrary,

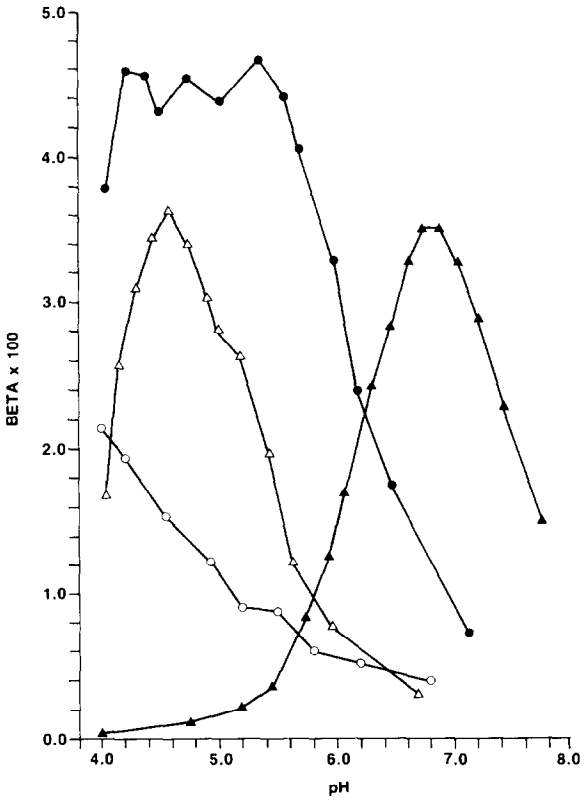

Fig. 1. In vitro buffer capacity versus pH profile for lacrimal fluid and the test formulations. Lacrimal fluid, ○; acetate-buffered, △; phosphate-buffered, ▲, citrate-buffered, ●.

the buffer capacities of the acetate- and citrate-buffered formulations at pH 4 were higher than the buffer capacity of the tears. Therefore, instillation of these formulations overwhelmed the buffer capacity of the tears (ΔpH 3.5 units).

Fig. 1 also illustrates some of the physicochemical differences between the buffer systems evaluated. For instance, the buffer capacity of the citrate-buffered formulation remained high over the entire pH re-equilibration range due to its proximally located ionization constants. The buffer capacity of the acetate formulation peaked at pH ~ 4.5 corresponding to its pK_a, but fell rapidly with increasing pH. Conversely, the phosphate-buffered formulation and negligible buffer capacity at pH 4, but became an increasingly stronger buffer at near-physiological pH. Based on these observations it was predicted that the citrate-buffered formulation would both depress the tear film pH and also slow down the pH re-equilibration rate. The acetate-buffered solution would cause a substantial initial drop tear pH without impeding subsequent pH re-equilibration. Finally, the phosphate-buffered formulation was expected to cause a smaller but sustained drop in post-instillation tear pH. These predictions proved to be valid as judged from the actual in vivo tear pH profiles (Fig. 2). It was noted that the pH following instillation of the unbuffered solution equilibrated rapidly and attained the physiological value within 4 min, while the acetate-buffered solution required 10 min. Although the initial rate of change in pH in the precorneal area were similar for the acetate- and phosphate-buffered

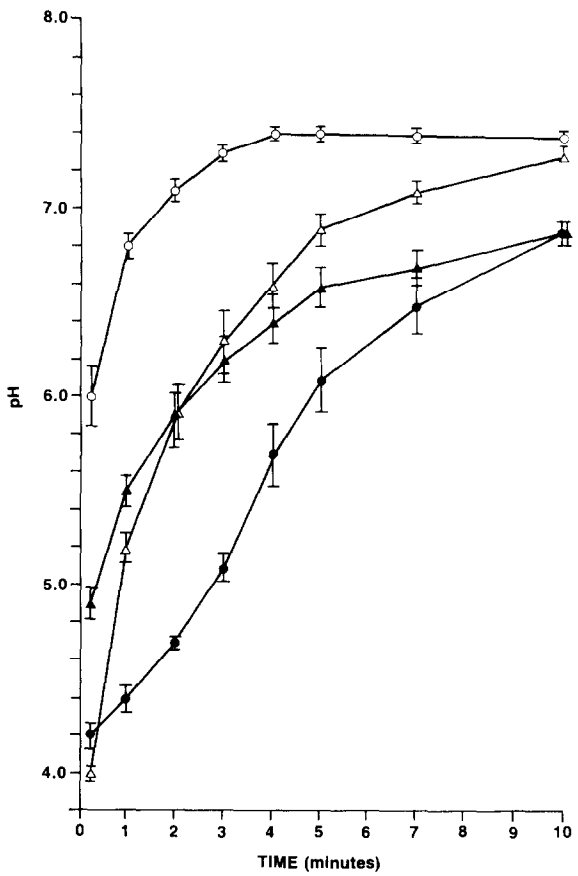

Fig. 2. In vivo tear pH profiles following instillation of the test formulations in rabbit eye. Unbuffered, ○; acetate-buffered, △; phosphate-buffered, ▲; citrate-buffered, ●.

solutions, a substantial reduction in the re-equilibration rate was observed in case of the latter as the tear pH began to rise. The citrate-buffered formulation yielded consistently lower pH values. The pH re-equilibration profile showed two inflection points – one at pH ~4.5 and the other at ~6.0 – corresponding closely to the pK_2 and pK_3 values for citrate.

Clearly, in an environment of changing pH as seen in the precorneal area after solution instillation, it is not adequate to compare buffer systems at a unique pH value. To overcome this problem, a new buffer parameter termed the "average buffer capacity (β_{avg})" was introduced to take into account the buffer capacity over the entire pH range of interest. β_{avg} was defined as the area under the buffer capacity versus pH profile:

$$\beta_{avg} = \int_{pH\ 4.0}^{pH\ 7.4} \beta_{expt} \cdot dpH \qquad (1)$$

From the calculated β_{avg} values listed in Table 1 it is noted that the strongest "effectively" buffered formulation contained citrate. Although acetate was a much stronger buffer than phosphate at pH 4.0, the average buffer capacities of the two systems were comparable over the pH range 4–7.4.

Topically instilled drugs typically suffer poor ocular bioavailability due to the presence of an efficient precorneal drainage mechanism (Chrai et al., 1973). A favorable pH environment in the precorneal area may improve ocular drug bioavailability by minimizing pH-induced lacrimation (Conrad et al., 1978). Rapid pH re-equilibration in the tears is considered to be additionally important for pilocarpine in order to convert the molecule from a poorly absorbed ionized form in the dosage form, to a preferentially absorbed unionized form (Francoeur at al., 1983) in the precorneal area. The reduction in ocular bioavailability of pilocarpine from buffered, acidic solutions has been attributed primarily to a pH-partitioning effect (Mitra and Mikkelson, 1982) and secondarily to pH-induced lacrimation (Ahmed and Patton, 1984). It is thus important to examine how buffers affect precorneal drug disposition and pH, and utilize this information to predict dosage form efficacy. In order to examine the buffer effect on precorneal fluid dynamics and drug disposition, inulin and pilocarpine concentrations in the tear chamber were measured after instillation. Since the concentration of inulin and pilocarpine in the tears were found to decline monoexponentially as previously reported (Patton and Robinson, 1976; Ahmed and Patton, 1984), first-order rate constants were obtained from the slope of the log concentration versus time plots. The apparent first-order rate constants for precorneal loss of inulin and pilocarpine from the 4 test formulations are given in Table 2.

TABLE 2

Apparent first-order rate constants for precorneal loss of inulin and pilocarpine

Formulation	Rate constant (min^{-1})		r
	Inulin	Pilocarpine	
Unbuffered	0.48 (0.070; 6) [1]	0.66 (0.097; 6)	0.961–0.995
Phosphate-buffered	0.54 (0.052; 6)	0.63 (0.075; 6)	0.972–0.990
Acetate-buffered	0.68 (0.041; 6)	0.77 (0.070; 6)	0.971–0.998
Citrate-buffered	0.58	0.64	0.975–0.998

[1] The standard error and sample size.

Although inulin has been found to be absorbed into the eye (Longwell et al., 1976), its rate of absorption is slow and its extent of absorption is small (Ahmed and Patton, 1985). Thus, the precorneal kinetics of inulin is primarily a measure of precorneal fluid dynamics, i.e. the rate of drainage, tear turn-over and the extent of induced lacrimation. It has been reported that a solution of non-physiological pH can induce lacrimation and lead to a faster rate of drug loss from the precorneal area (Conrad et al., 1978). The fastest decline in inulin concentration was observed in the acetate-buffered formulation, followed by the citrate, phosphate and the unbuffered formulations, respectively (Table 2). With the exception of the acetate, the results indicated that the extent of induced lacrimation correlated with the extent and duration of pH depression in the precorneal area. However, the disproportionately high lacrimation

in case of the acetate-buffered formulation is difficult to reconcile in terms of pH effects alone. It is likely that acetic acid or the acetate ion species is inherently more irritating to the eye than the other buffers examined. Instillation of the acetate buffered, pH 4.0, solution in the rabbit eye also caused noticable discomfort and conjunctival vasodilation (redness).

The precorneal kinetics of the buffer species were not quantitated directly. However, considering the fact that in normal , conscious rabbits precorneal loss due to fluid dynamics predominates over drug loss due to ocular absorption (Lee and Robinson, 1979), and that the extent of ocular absorption of the buffers is likely to be small, the precorneal buffer concentrations were equated to those of inulin in the corresponding formulations. Accordingly, the precorneal rate constants obtained for inulin were used to predict the total buffer concentration in the tears as a function of time after instillation. This information, in combination with the lacrimal fluid pH–time profiles, were used to calculate residual buffer capacity, β_t:

$$\beta_t = \sum 2.303 K_a [H^+(t)][C(t)] \Big/ (K_a + [H^+(t)])^2 \qquad (2)$$

where K_a denotes the buffer dissociation constant, $[H^+(t)]$ the observed hydrogen ion concentration in the tears at time t post-instillation and $C(t)$ the residual buffer concentration at time t post-installation. Residual buffer capacity plots generated as shown in Fig. 3, indicate the buffer effect that persists in the precorneal area as a function of time. Since buffer capacity is a function not only of buffer concentration, but depends also on the ionization constant and pH, a buffer may maintain a substantial buffering effect even at very low concentration. This is graphically apparent in the case of the phosphate-buffered solution. Although the concentration of phosphate in the tears declines continually after solution instillation, its buffer capacity actually increases as the tear pH rises towards the pK_2 of phosphate. This observation lends further credibility to the explanation

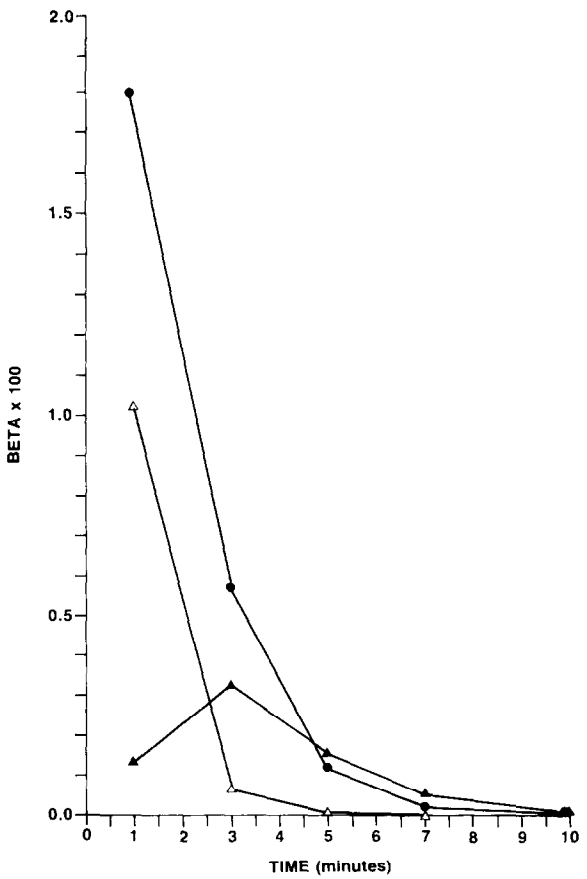

Fig. 3. Simulated residual buffer capacity versus time plots for the test formulations. Acetate-buffered, △; phosphate-buffered, ▲; citrate-buffered, ●.

given earlier for the deceleration in the pH re-equilibration rate caused by the phosphate-buffered formulation. Therefore, both precorneal fluid dynamics, and the concentration and type of buffer present in the instilled formulation, influence the time course of lacrimal fluid pH.

Assessment of relative drug absorption efficiency from ophthalmic dosage forms may be based on: (1) precorneal rate constant analysis (Patton and Robinson, 1976; Ahmed and Patton, 1984); (2) estimation of area under the tear drug concentration versus time curves (Maurice and Mishima, 1984); (3) aqueous humor and intraocular tissue drug levels (Tang-Liu et al., 1984; Patton and Francoeur, 1978); and (4) relative pharmacological response (Mitra and Mikkelson, 1983). Ap-

proaches (1) and (2) are indirect, rely basically on quantitation of precorneal variables, comprised of non-invasive techniques, and are less time- and labor-intensive. Approaches (3) and (4) provide a direct measure of ocular drug bioavailability, information regarded essential for the final dosage form efficacy and safety evaluation. However, during the initial stages of product development and formulation screening, the direct approaches may prove to be excessively time-, labor- and resource-intensive. In these cases, it may suffice to use the indirect approaches. The applicability and utility of approaches (1) and (2) for screening buffer systems and predicting the relative absorption efficacy of pilocarpine from different formulations were evaluated in some detail in this study. Pilocarpine bioavailability in the eye based on area under miosis–time profiles (approach 4) was also determined for reference.

As noted previously, the decline in inulin concentration in the tear chamber is attributed primarily to fluid dynamics. Conversely, the decline in pilocarpine concentration is due to both fluid dynamics and ocular absorption (Table 2). Since the formulations evaluated were identical in their drug composition, the difference in rate constant between pilocarpine and inulin provides a measure of the apparent absorption rate for pilocarpine (Ahmed and Patton, 1984). Due to the short length of time over which ocular absorption of pilocarpine occurs (Sieg and Robinson, 1976), the apparent absorption rate calculated from the initial tear drug concentration data has been shown to correlate well with intraocular pilocarpine levels (Ahmed and Patton, 1984). The rank order of absorption efficiency predicted by this approach was: unbuffered > phosphate-buffered ≃ acetate-buffered > citrate-buffered.

It is clear that for drugs absorbed passively into the eye, the drug concentration in the tear serves as the driving force for transport (Thombre and Himmelstein, 1984). The area under the tear concentration versus time profile may then be regarded as a measure of the "absorbable" dose and the absorption potential for the drug from a particular dosage form. For pilocarpine, the scenario is complicated by the fact that the drug exists in the tears in either an ionized or an unionized form of vastly different permeabilities (Francoeur et al., 1983). It is predominantly the unionized species that contributes to intraocular levels of pilocarpine. However, the tear concentrations of pilocarpine measured in this study reflected total drug and not its ionization status. Obviously, the area under the total tear drug concentration profile would not serve as a representation of the true absorption potential for pilocarpine. Fortunately, with the data on the time course of lacrimal fluid pH for each formulation already available, the total concentration can be converted to unionized drug concentration using the equation:

$$C_u = C_t 10^{-K_a} / (10^{-K_a} + [H^+]) \qquad (3)$$

where, C_u is the unionized concentration, C_t the total measured concentration, K_a the ionization constant for pilocarpine ($pK = 6.88$) and $[H^+]$ the hydrogen ion concentration in the tears. The C_u versus t profiles for the 4 formulations are shown in Fig. 4. The rank order in the area under the curve (AUC) was: unbuffered > acetate-buffered > phosphate-buffered > citrate-buffered.

Finally, pilocarpine is known to cause miosis or pupil constriction. The extent of this pharmacological effect is related to drug concentration in the iris-spincter muscle (Smolen and Schoenwald, 1971) and has commonly been used as a measure of the ocular bioavailability of pilocarpine and other drugs. The average observed changes in pupillary diameter as a function of time are shown in Fig. 5. The rank order in AUC was: unbuffered > acetate-buffered > phosphate-buffered > citrate-buffered.

The parameters for absorption efficiency obtained by the different approaches are summarized in Table 3. The results based on tear concentration (C_u) were in agreement with the results based on ΔPD–time curve both in terms of rank order and relative absorption efficiency. It is noted that the absortion efficiency of pilocarpine from the phosphate-buffered formulation was less than from the acetate-buffered formulation, although the acetate-containing formulation was more strongly buffered at the pH formulated and resulted in more lacrimation. This finding was also reported in a recent study where the investigators

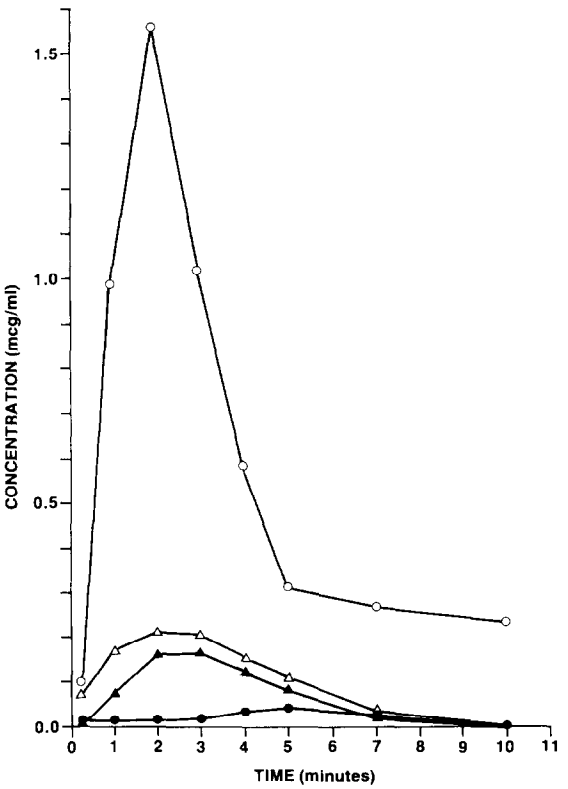

Fig. 4. Simulated unionized pilocarpine tear concentration profiles following instillation of the formulations. Unbuffered, ○; acetate-buffered, △; phosphate-buffered, ▲; citrate-buffered, ●.

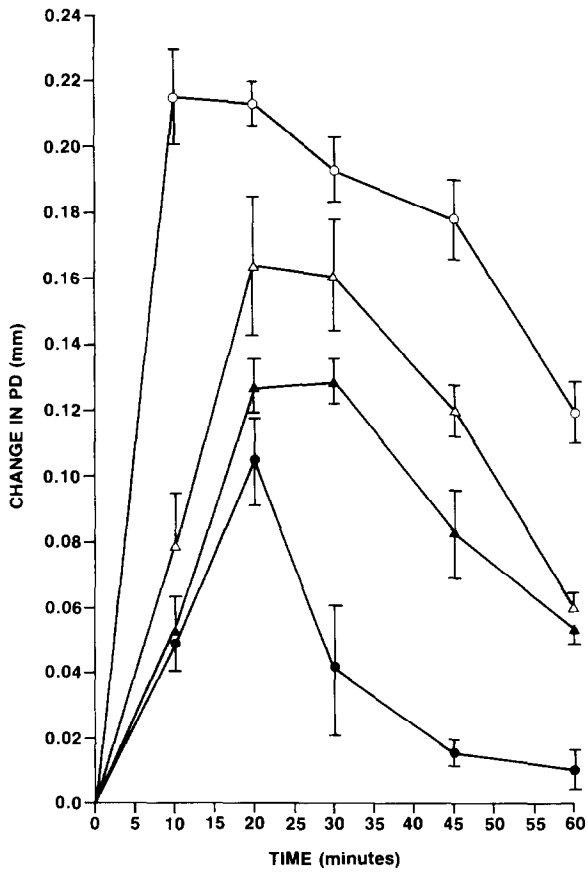

Fig. 5. Miosis–time profiles: average changes in pupillary diameter after instillation of the test formulations. Unbuffered, ○; acetate-buffered, △; phosphate-buffered, ▲; citrate-buffered, ●.

postulated phosphate exerting a resistance to pH change near the pK_a of pilocarpine, thereby trapping it in the less bioavailable ionized form (Mitra and Mikkelson, 1987). The hypothesis was proven to be valid in the present study as evident from the time course for pH re-equilibration for the different buffer systems in the tears. Finally, the results obtained from precorneal rate constant analysis were consistent with actual in vivo findings in regard to the relative absorption efficiency of pilocarpine from the different formulations. However, the error in determination of the absorption rate constant and the inherent assumptions involved diminish the reliability and accuracy of the procedure in predicting relative ocular availability in some cases.

In conclusion, buffer properties which dictate the absorption efficiency of pilocarpine are the concentration and type of buffer used. The functionality and the value of the buffer pK_a relative to the pK_a of the drug is critically important in case of pilocarpine because the drug undergoes a change in its ionization status within the pH range

TABLE 3

Parameter estimates for absorption efficiency of pilocapine

Formulation	Apparent absorption rate constant (min^{-1})	AUC C_u vs t ($\mu g/\mu l \cdot min$)	AUC ΔPD vs t (mm × min)
Unbuffered	0.18	5.10	15.9
Phosphate-buffered	0.09	0.72	5.84
Acetate-buffered	0.09	1.05	7.29
Citrate-buffered	0.06	0.31	2.32

of interest. Rapid pH re-equilibration is essential for optimum pilocarpine activity. The manner in which buffers dictate the time course of pH and drug disposition in vivo can be adequately predicted based on physicochemical parameters obtained in vitro. Finally, quantitation of precorneal variables such as the time course of tear pH and precorneal drug concentration can be used to predict relative absorption efficiency of pilocarpine. These approaches described do not require the use of complicated mathematical models and can be easily incorporated in the protocol for ophthalmic preformulation studies.

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