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Ophthalmic solution buffer systems. II. Effects of buffer type and concentration on the ocular absorption of pilocarpine and a method of ocular bioavailability prediction from physicochemical data

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Summary

The objective of the study has been to quantitatively determine the effects of the concentrations and types of buffer used in ophthalmic drug solutions or formulations on the corneal absorption efficiency or ocular bioavailability of a representative amine drug, pilocarpine. The ophthalmic solutions containing pilocarpine (as nitrate or hydrochloride salts) are buffered in the pH range of 4.5–5.0 for reasons of optimum chemical stability. But such a pH range causes only a smaller fraction of total pilocarpine (pK_a of protonated base 6.85 at 25 °C) to be available as preferentially absorbed unionized species. Since tear fluid has a very low buffer capacity, ophthalmic solutions containing buffers of high buffer capacities within the pH range of 4.75–7.40 would tend to resist the pH alteration of the instilled fluid by tear fluid to a greater degree and consequently would lower the extent of drug absorption. Miosis–time profiles were obtained following the instillation of 25.0 μl of isotonic 1.0% (w/v) pilocarpine nitrate solutions which were buffered at a pH of 4.75 with the same initial 0.075 M concentration of citrate, phosphate or acetate buffers. Relative pharmacological response, as measured by the areas under the miosis–time profiles (AUC) and the maximum observed pupillary diameter changes (ΔPD_{max}), decreased in the order of: no buffer > acetate > phosphate > citrate. These isotonic buffer solutions containing 1% (w/v) pilocarpine nitrate were titrated in vitro with a standard base and the volume of 1.0 N sodium hydroxide needed to titrate the drug solutions from a pH of 4.75 to 7.40 showed excellent inverse linear relationships with the ocular bioavailability parameters. The correlations developed appear to have the capability of predicting relative ocular bioavailabilities of pilocarpine solutions as a function of buffer concentration for citrate, phosphate and acetate buffer systems.

Introduction

It was previously shown (Mitra and Mikkelson, 1982) that increasing the concentration of citrate buffer in pilocarpine eye drops reduced the extent of corneal absorption of the drug as measured by the maximum change in pupillary diameter or the area under the miosis–time curve. Greater than 5-fold reduction in the miosis–time curve was noted at the highest citrate buffer concentration (0.11 M). Furthermore, Keller et al. (1980) reported that a well buffered pH 4 solution could depress tear pH for about 10–15 min due to very low buffer capacity of the tear fluid. Considering

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the limited time (5 min) over which corneal absorption occurs (Sieg and Robinson, 1976), lowering of tear pH can result in a greater fraction of pilocarpine being available as poorly absorbable ionized pilocarpine species. The permeability of pilocarpine ions across the cornea is only 4.82×10^{-6} cm · s^{-1} whereas that of the unionized free base species is about two times, 9.74×10^{-6} cm · s^{-1} at 34°C (Mitra, 1983). Therefore, the buffer capacity of the instilled solution can dictate the time course of pH, drug ionization and hence the extent of pilocarpine absorption.

Ophthalmic solutions containing amine drugs of therapeutic application in glaucoma such as pilocarpine and epinephrine require buffering in the acidic pH region for reasons such as chemical stability and/or solubility. However, the physicochemical demands are met at the expense of absorption efficiency or ocular bioavailability of these amines because they are essentially delivered as less permeable ionized species. It is the purpose of our studies to quantitatively evaluate the effects of buffer types and functionality (mono, bi, etc.) on the ocular absorption of pilocarpine. Specifically the effects of 0.075 M citrate, phosphate and acetate buffers on the efficiency of ocular absorption of isotonic 1.0% w/v pilocarpine nitrate solutions, have been evaluated in New Zealand albino rabbits following topical instillation.

Materials and Methods

Materials

Pilocarpine nitrate was obtained from Sigma Chemicals, St. Louis, MO. All other chemicals used were of analytical reagent grade.

Drug solutions

The drug solutions for topical application were aqueous isotonic 1.0% w/v pilocarpine nitrate solutions buffered at pH 4.75 with 0.075 M acetate, citrate or phosphate buffers. The solutions differed only in the buffer types and sodium chloride contents. The solutions were prepared by dissolving pilocarpine nitrate and the required amount of sodium chloride in appropriate volumes of combinations of stock solutions of buffer components in water. Sodium chloride was added in amounts necessary to render the various buffered pilocarpine solutions iso-osmotic with physiological fluids. The amount of sodium chloride necessary for each formulation was calculated by the freezing point depression method using literature L_{iso} values for drug, buffer components and sodium chloride. The test solutions were not sterilized, but were freshly prepared immediately prior to use.

Miosis–time profiles

Adult male albino rabbits (New Zealand strain) were the experimental animals. The animal weights were 4.2 ± 0.3 kg with a range of 3.9–4.5 kg. No particular animal pretreatment procedures regarding water, diet or environment were followed. The miosis–time profiles were obtained as described previously (Mikkelson et al., 1973). Pupillary diameter measurements were made using a cathetometer (Eberback, Ann Arbor, MI) with an accuracy of ± 0.1 mm. The same individual was responsible for all pupillary diameter measurements. The test animals were unanesthetized, therefore normal precorneal fluid dynamics remained unaltered. The studies were conducted in an isolated and constant environment to minimize auditory and visual stimuli. Drug solution was instilled as 25 μl fluid volume using a lambda pipette. A minimum of one week wash out time period between experiments in a single animal (either eye) was allowed because it was predetermined that such period of time ensured the absence of a carry-over effect or contribution to the observed pharmacological response. The normal baseline or pretreatment pupillary diameters were 6.9 ± 0.4 mm with a range of 6.5–7.3 mm.

Buffer capacity calculation

A buffer solution contains an appreciable concentration of a weak acid or base and its salt. The addition of a small quantity of acid or alkali to such a buffer solution would cause a small shift in pH as compared with that which would be observed if the acid or base were added to water. The higher the buffer capacity or index of a solution at a given pH, the more resistance it offers to change in pH following acid or alkali addition. The optimum buffer capacity of a buffer

is at the pH = pK_a for the weak acid or base. At 1 pH unit away from the pK_a, a buffer is about 33% as effective.

Buffer capacity or index values were calculated β_{cal}, as described by Butler (1964). The total buffer capacities of the formulations are contributions not only from the mono- (acetate) or polyfunctional (phosphate and citrate) buffer systems but also from the monofunctional acidic pilocarpinium ion. For systems containing mono and polyfunctional buffers, where successive pK_a values differ by greater than 1.3 units and the system is not buffered at either extreme of the pH scale, the following equation (Butler, 1964) estimates buffer capacity within ±5.0%.

$$\beta_{cal} = \sum_{i=1} 2.303 \frac{K_{a,i}[H^+][C_i]}{(K_{a,i} + [H^+])^2} \quad (1)$$

where the subscript, i, represents each component or ionization contributing to the total buffer capacity. $[C_i]$ denotes the molar concentration of each species, K_a is the acid dissociation constant for each individual component and $[H^+]$ is molar hydrogen ion concentration.

In vitro titration of ophthalmic formulations

Six different isotonic solutions of 1.0% pilocarpine nitrate were prepared containing none, 0.055, 0.075 and 0.11 M citrate, 0.075 M phosphate and 0.075 M acetate. All solutions were buffered initially to a pH of 4.75. Ten ml volumes of each formulation were titrated by 50 μl incremental addition of 1.0 N sodium hydroxide to a final pH of 7.40. The pH was recorded after each addition and the total volume of titrant was noted following each addition.

Results and Discussion

The present studies were undertaken to quantitatively evaluate the effects of various buffer types on the efficiency of ocular absorption of pilocarpine. Commercial ophthalmic solutions containing pilocarpine (as the nitrate or hydrochloride salt) are buffered in the 4.5–5.0 pH range for reasons of chemical stability. The experimental drug solutions in the current studies were prepared using 0.075 M acetate, phosphate and citrate buffers. Therefore, the drug solutions contained the same concentration of pilocarpine nitrate (1% w/v) and buffer. The pH of the instilled solutions was chosen to be 4.75, which is the median of the allowed compendial limits (pH 4.0–5.5) for pilocarpine hydrochloride and pilocarpine nitrate ophthalmic solutions, U.S.P. XX.

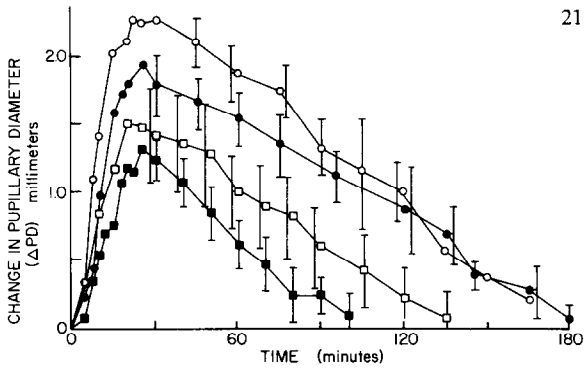

Fig. 1. Miosis–time profiles: plots of the mean observed changes in pupillary diameter (ΔPD) as a function of time following the instillation of 25.0 μl of the isotonic 1.0% w/v pilocarpine nitrate solutions, which contained the same 0.075 M concentration of various buffers. ○, no buffer; ●, acetate; □, phosphate; ■, citrate. The vertical lines through the data points are ±S.D. (Data points with standard deviation lines omitted is for clarity of the figure.)

Fig. 1 illustrates the plots of the mean observed changes in pupillary diameter (ΔPD) as a function of time following the instillation of 25.0 μl of isotonic 1.0% w/v pilocarpine nitrate solutions, which contained the same concentrations (0.075 M) of different buffers (acetate, citrate and phosphate) adjusted to an initial pH of 4.75. Differences in pharmacological activity as measured by the miotic response to the drug are apparent. The general shapes of the miosis–time profiles are similar and the apparent elimination phases from all 4 profiles suggest similar elimination behavior. However, the magnitude of miotic response to pilocarpine and the duration of miotic effect were significantly altered by the type or functionality of the buffer used in the formulation. The miotic activity or relative pharmacological response due to 1% w/v pilocarpine decreased in the rank order of no buffer > 0.075 M acetate > 0.075 M phos-

TABLE 1

Maximum observed pupillary diameter change ($\Delta PD\_$), area under the miosis–time profile (AUC), and their relative values for the citrate, phosphate and acetate buffered 1% (w/v) pilocarpine nitrate solutions (25 μl) administered topically to the rabbits

Buffer constituent	None	Acetate	Phosphate	Citrate
Total molar concentration of buffer	0	0.075	0.075	0.075
n	6	6	6	6
ΔPD_{max} (mm)	2.45 ± 0.23	1.98 ± 0.64	1.48 ± 0.36	1.35 ± 0.08
ΔPD_{max}^{rel}	1.0	0.81	0.60	0.55
AUC (mm · min)	224 ±22	180 ±22	111 ±31	73 ±14
AUC^{rel}	1.00	8.60	0.50	0.33

The values are expressed as mean ± S.D. n = number of determinations. ΔPD_{max} = maximum observed change in pupillary diameter. ΔPD_{max}^{rel} = relative values of ΔPD_{max} using zero buffer concentration as the reference. AUC = area under the miosis–time profile curve. AUC^{rel} = relative AUC using zero buffer concentration as the reference.

phate > 0.075 M citrate. As a control, instillation of buffer solutions without any drug resulted in no pupillary response.

The experimentally observed differences in the miosis studies were quantitated by typical methods. Both the maximum change in pupillary diameter, ΔPD_{max}, which could be observed from Fig. 1, to be occurring within 20–30 min post-instillation, and the areas under the miosis–time profile curves, AUC, which were estimated using the trapezoidal method for the individual experiments with each of the buffers have been calculated for each experiment. The averaged values of ΔPD_{max} and AUC for each of the buffers used are given in Table 1.

The relative ocular bioavailabilities as measured by AUC^{rel} appear to decrease from 1.0 to 0.80 for the acetate to 0.50 for the phosphate and finally to 0.33 for the citrate buffer systems. When the ΔPD_{max}^{rel} values are compared, similar rank order correlation is found among the 3 buffer systems and the magnitude of differences are also similar. ΔPD_{max}^{rel} values decreased to 0.81 for acetate buffer systems, to 0.60 for phosphate buffer systems and to 0.55 for the citrate buffer systems. Statistical significance tests in the manner of one-way analysis of variance, were conducted on the AUC and ΔPD_{max} data presented in Table 1. In the AUC data, all of the possible 6 comparisons showed statistical significance at the 95% confidence level, whereas, in the ΔPD_{max} data for the 6 possible comparisons, only one (0.075 M phosphate versus 0.075 M citrate) did not show statistically significant difference at the 95% confidence level.

The studies performed with the same initial concentration of buffers (0.075 M), drug concentration (1% w/v) and initial pH (4.75), produced significantly different ΔPD_{max} and AUC values which suggests that the buffer type or functionality can affect the extent of ocular absorption of an amine drug like pilocarpine. Sieg and Robinson (1976, 1977) have pointed out that the normal or resident tear volume is about 7.5 μl in the rabbit, with a normal tear turnover rate of 0.7 μl/min. Instillation of excess fluid such as introduction of a drug solution into the precorneal space results in rapid removal of the introduced fluid back to the normal volume. This process of volume dependent drainage of an instilled solution is essentially complete within 5 min of dosing. The initial rapid loss of an instilled drug solution infers that a high precorneal drug concentration, necessary and responsible for productive corneal absorption, is of short duration. Therefore, the ionization state of drug molecules, i.e. ratio of highly permeable unionized species to relatively less permeable ionized species can have significant effect on the relative absorption efficiency of an ionizable drug. Upon instillation of a 25 μl volume of a 1.0% buffered solution of pilocarpine nitrate, which is buffered at a pH of 4.75, into the precorneal space, the solution will undergo mixing with the lacrimal or tear fluid. Since the tear fluid

has very little buffer capacity, the pH of the precorneal fluid would probably be depressed to or near the instilled solution pH. Keller et al. (1980) reported that a well buffered pH 4 solution depressed tear pH for 10–15 min. But the pH of the precorneal fluid will gradually rise because of the continual removal of buffer mixed precorneal solution coupled with constant titration of the precorneal fluid by pH 7.4 tear fluid. The precorneal fluid dynamics and the concentration and type of buffer present in the formulation will dictate the change in pH of the precorneal fluid. The magnitude of increase in pH due to titration by tear fluid, and the rate at which the process occurs, changes the degree of ionization of the amine drug instilled into the precorneal space. The more the acid–base equilibrium is adjusted toward the free base, the more rapid and efficient total absorption of the amine will result. As it has already been stated that the time over which ocular absorption can occur before an instilled drug solution is completely removed by physiological mechanisms is quite short, the type and concentration (capacity or index) of buffer contained in the instilled fluid plays a significant role in the absorption efficiency or ocular bioavailability. Our previous work with citrate buffer systems (Mitra and Mikkelson, 1982) have shown that a gradual increase in citrate buffer concentration causes a corresponding decrease in ocular absorption of pilocarpine. Ultimately, 5-fold reduction in the area under the miosis–time profile curve was obtained with the highest concentration (0.11 M) of citrate used. The higher the concentration of buffer contained in the instilled solution, the more resistant the solution would be to change in pH by the lacrimal fluid with which it is mixed.

The differences in ocular bioavailability observed with the solutions containing same initial buffer concentrations (0.075 M) and same starting pH (4.75) could be explained by examining the buffer capacity profiles of each of the buffers within the pH range of interest, i.e. from pH 4.75 to 7.40. Such buffer capacity profiles have been illustrated in Fig. 2A, B and C. The plots depict the change in buffer capacity as a function of pH for acetate, phosphate and citrate buffer systems, respectively. It must be recognized that pH change of one unit around the pK_a causes the most significant change (90%) in unionized-to-ionized ratio. Therefore, buffer substances having pK_as closer to the pilocarpine pK_a would be most effective in resisting the pH change near the pK_a of pilocarpine exerting maximum inhibitory effect on the formation of more bioavailable unionized pilocarpine free base species. Examination of buffer capacity profiles depicted in Fig. 2A, B and C clearly suggests that acetate being a monofunction buffer with a pK_a of 4.75 has very little buffer capacity beyond pH 6.0 and the only buffer capacity contribution near pilocarpine pK_a is from the drug itself. Phosphate, on the other hand because of its second pK_a of 6.88, will offer significantly higher resistance to pH change near the pilocarpine pK_a. The total buffer capacity due to

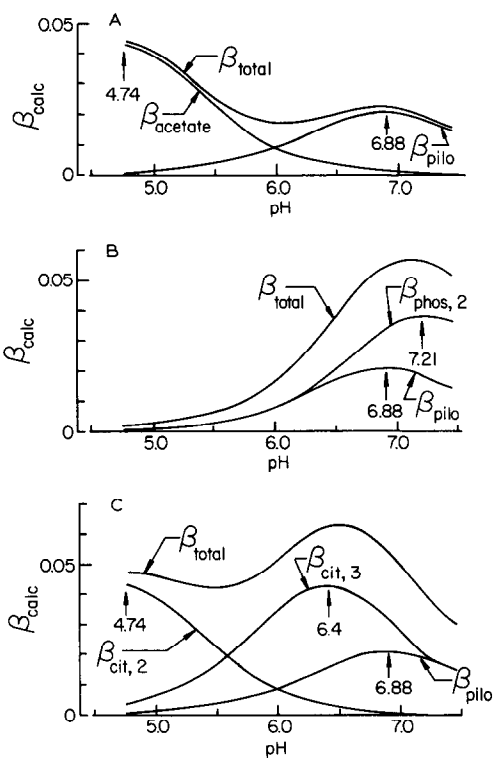

Fig. 2. Plots of calculated buffer capacities (β_{cal}) as a function of pH over the pH range of 4.75–7.40 for 3 different buffer systems: (a) acetate; (b) phosphate; (c) citrate. The subscripts acetate, phos, cit, pilo and total refer to the buffer capacity contributions from acetate phosphate, citrate, pilocarpine and the total species, respectively. The particular pK_a value for each of the species involved has been shown.

citrate buffer and the drug is considerable (0.05 or greater) throughout the entire pH range from 4.75 to 7.40 because of the participation of two citrate pK_as (pK_as of 4.74 and 6.4). Therefore, the citrate buffer will resist any pH change of the precorneal fluid by lacrimal fluid over the entire pH range of 4.75–7.40. From the buffer capacity calculations it is clear that acetate offers the least amount of buffer capacity, phosphate the intermediate and citrate the most within the pH region of interest. As a result, the order of ocular bioavailability of pilocarpine buffered at pH 4.75 with 0.075 M concentration of each of these three buffers should be in the order of: acetate > phosphate > citrate, a hypothesis which is in excellent agreement with the experimentally obtained pharmacological response data presented in Table 1.

In order to understand the quantitative nature of the interaction between the buffer concentration and/or type and the ocular absorption efficiency of pilocarpine, a workable in vitro–in vivo correlation has been developed. In vitro buffering strength was determined by performing titrations of each of the 6 formulations containing 1.0% w/v pilocarpine nitrate. Ten ml volumes of drug solution containing various buffers were titrated from

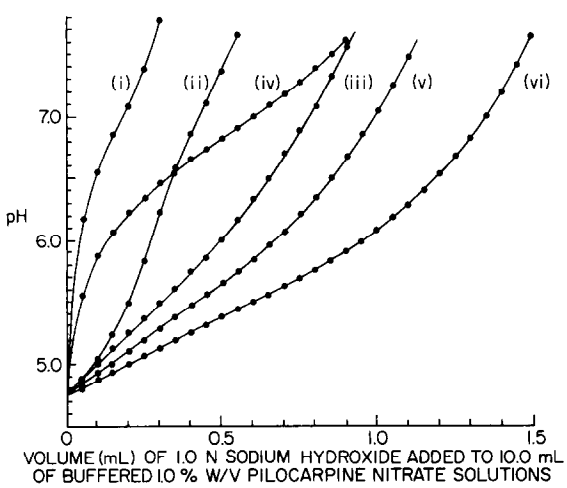

Fig. 3. pH–titration profiles of a series of formulation of 1.0% w/v pilocarpine nitrate containing different concentrations of various buffers. The formulations were titrated from a starting pH of 4.75 to a final pH of 7.40 by incremental additions of 1.0 N sodium hydroxide. (i) no buffer; (ii) 0.075 M acetate; (iii) 0.055 M citrate; (iv) 0.075 M phosphate; (v) 0.075 M citrate; (vi) 0.11 M citrate.

an initial pH of 4.75 to a final pH of 7.40 with successive additions of 50 μl volumes of 1.0 N sodium hydroxide titrant. The solution pH was recorded following each titrant addition. Fig. 3 is such a plot of pH as a function of the volume of titrant added for each of the 6 formulations labelled by roman numerals (i) through (vi). As expected, for the same molar concentration (0.075 M) of the 3 buffers, acetate required the least amount, phosphate the intermediate and citrate the most amount of 1.0 N base to be titrated from pH 4.75 to pH 7.40. Moreover, as the citrate buffer concentration was increased from 0.055 to 0.11 M, a correspondingly higher quantity of base was required to titrate the solution.

The in vivo data are biological response parameters (AUC and ΔPD_{max}). Biological response and the corresponding drug concentration are typically related and can be linearized by log–log plots as described in Eqn. 2 (Gibaldi and Perrier, 1982)

$$\log \frac{E}{E_{max} - E} = S \log C + \log Q \qquad (2)$$

where E is the response intensity, E_{max} is the maximum response intensity (of a reference formulation), C is the available concentration of the drug producing the pharmacological response (absorbed dose) and S and Q are constants.

Since the drug concentration available to produce pharmacological response can be inversely related to the buffer capacity as expressed by the volume of 1.0 N base titrant needed to titrate the drug solution from the initial pH of 4.75 to a final pH of 7.40 ($V_{7.4}$), the term C can probably be replaced by $(V_{7.4})^{-1}$ in Eqn. 2. This inverse relationship stems from the fact that the larger the volume of titrant required to titrate the buffered drug solution, greater resistance to pH change would be expected to occur resulting in smaller fraction of pilocarpine as the more absorbable free base species in the precorneal fluid.

Using area under the miosis–time profile curves (AUC) as the response parameter, E, and substituting $(V_{7.4})^{-1}$ for C in Eqn. 2.

$$\log \frac{AUC}{AUC_{max} - AUC} = S \log (V_{7.4})^{-1} + \log Q \qquad (3)$$

TABLE 2

Titration volumes and pharmacological response parameters for 1% (w/v) pilocarpine nitrate solutions containing various buffers

Solution no.	Buffer system	$V_{7.4}$ (ml)	AUC (mm · min)	$\frac{AUC}{AUC_{max} - AUC}$	ΔPD (mm)	$\frac{\Delta PD}{\Delta PD_{max} - \Delta PD}$
(i)	None	0.25	224 ± 22	–	2.45 ± 0.23	–
(ii)	0.075 M acetate	0.51	180 ± 22	4.09 ± 3.53	1.98 ± 0.04	4.21 ± 0.45
(iii)	0.055 M citrate	0.87	124 ± 24	1.24 ± 0.57	1.78 ± 0.23	2.66 ± 1.65
(iv)	0.075 M phosphate	0.81	111 ± 31	0.98 ± 0.59	1.48 ± 0.36	1.53 ± 1.46
(v)	0.075 M citrate	1.09	73 ± 14	0.48 ± 0.14	1.35 ± 0.08	1.23 ± 0.16
(vi)	0.110 M citrate	1.45	40 ± 14	0.22 ± 0.09	1.03 ± 0.25	0.73 ± 0.31

The pharmacological response data tabulated as mean ± S.D. $n = 6$.

The quantities $V_{7.4}$, corresponding AUC and ΔPD values and the quotients $AUC/(AUC_{max} - AUC)$ and $\Delta PD/(\Delta PD_{max} - \Delta PD)$ have been presented in Table 2.

A plot of the logarithm of $AUC/(AUC_{max} - AUC)$ versus logarithm of $(V_{7.4})^{-1}$ plot shows excellent linear behavior as depicted in Fig. 4. Linear regression of the data resulted in the following parameters, $n = 5$, $r = 0.989$, $S = 2.8$, $\log Q = -0.196$ or $Q = 0.640$, where n is the number of data points, r is the correlation coefficient and S and Q are constants.

AUC, the biological response parameter could also be replaced by ΔPD in Eqn. 3. A plot of logarithm of $\Delta PD/(\Delta PD_{max} - \Delta PD)$ versus logarithm of $(V_{7.4})^{-1}$ also shows good linearity as illustrated by Fig. 5. Linear regression of the data resulted in these parameters, $n = 5$, $r = 0.936$, $S = 1.65$, $\log Q = 0.155$ or $Q = 1.42$. Although different degrees of lacrimation induced by different buffer species can cause changes in pilocarpine availability, strong correlation among biological response parameters and reciprocal titrant base volumes suggest significant contribution from time course of ionization of pilocarpine in the precorneal fluid and the relative availability of ionized versus unionized pilocarpine species. The Eqn. 3

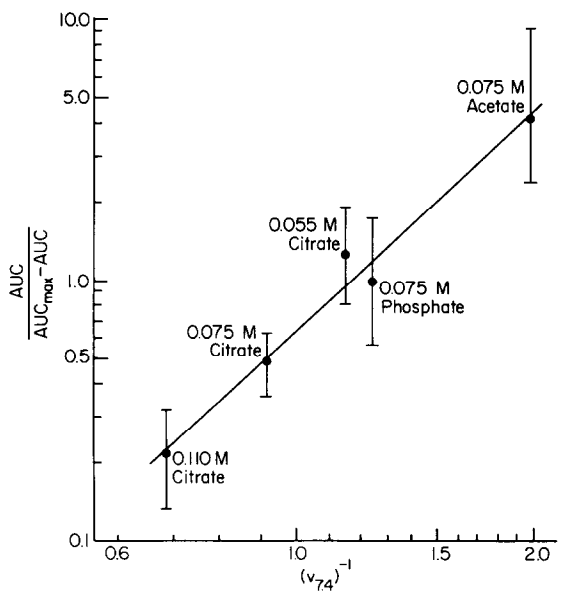

Fig. 4. In vitro–in vivo correlation. A plot of logarithm of the response parameter, $AUC/(AUC_{max} - AUC)$, as a function of the logarithm of the reciprocal of the volume of 1.0 N sodium hydroxide required to titrate the formulation $(V_{7.4})^{-1}$ from a pH of 4.75 to pH 7.40.

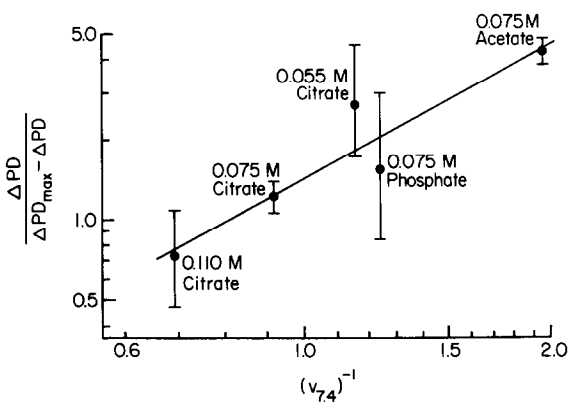

Fig. 5. In vitro–in vivo correlation. A plot of logarithm of the response parameter, $\Delta PD/(\Delta PD_{max} - \Delta PD)$, as a function of the logarithm of the reciprocal of the volume of 1.0 N sodium hydroxide required to titrate the formulation $(V_{7.4})^{-1}$ from a pH of 4.75 to pH 7.40.

that has been developed and the profiles that have been generated from that equation (Figs. 4 and 5), represent a strong correlation of an in vivo–in vitro nature. The equation has been formulated through utilization of known pharmacokinetic and pharmacodynamic principles, and it represents a means to predict what effect a buffer candidate for an ophthalmic formulation will have on the biological response or ocular bioavailability of the drug being delivered. The results of this study have significance both to investigators conducting studies in animals of an experimental or basic nature as well as to individuals involved in the formulation of commercial ophthalmic products.

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