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Ophthalmic solution buffer systems I. The effect of buffer concentration on the ocular absorption of pilocarpine

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Summary

Miosis–time profiles were obtained using rabbits following the instillation of 25.0 μl of 1.0% pilocarpine nitrate solutions, which were buffered at a pH of 4.75 with different concentrations of citrate buffer. Relative pharmacological response, as measured by the areas under the miosis–time profiles and the maximum observed pupillary diameter changes, decreased as the citrate buffer concentration was increased. A reduction in the miosis–time profile area of greater than 5-fold was observed at the highest buffer concentration studied. The study demonstrates that a non-drug formulation component, such as a buffer system, can dramatically affect the relative pharmacological response from a simple ophthalmic solution dosage form. These results are of importance both in basic ophthalmic research studies and in ophthalmic product research and development.

Introduction

The buffer systems contained in topical ophthalmic solutions and the pH to which they are adjusted have, at least qualitatively, long been recognized as being important relative to ocular drug absorption or activity. Gifford and Smith (1933) pointed out that both the ocular pharmacological and toxicological activity of amine drugs were greater when the acidity of the solutions used in their administration was adjusted to alkaline regions. These early observations (Gifford, 1933) are consistent

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with currently accepted mechanisms and theories of transport of ionizable drugs across biological membranes. Hind and Goyan (1947) discussed in qualitative terms significant aspects of the use of buffers in ophthalmic solutions. Included by them as important factors were solution pH and drug ionization. Studies directed at evaluation of the effect of ionization and pH as determinants in ocular drug activity have been reported by numerous investigators (Swan and White, 1942; Cogan and Hirsch, 1944; Cox et al., 1972a; Kupferman et al., 1974; Sieg and Robinson, 1977; Conrad et al., 1978). These studies have demonstrated that the pH and therefore the degree of ionization of a drug, plays a significant role in the efficiency of absorption in the eye. Results of these reported studies are not in disagreement with the pH-partition hypothesis in that the ionized form of an ionizable drug appears to undergo limited corneal transport in comparison to the unionized form.

Initially, Sieg and Robinson (1977) reported that the lower ocular pilocarpine bioavailability observed at lower instilled solution pH was not due to a specific pH-partition effect. Instead, they attributed it to induced lacrimation, the intensity of which increased as the pH of the instilled solution was adjusted to values away from physiological pH. This induced lacrimation resulted in a more rapid removal of the drug from the precorneal space via this induced nasolacrimal turnover mechanism. However, it was later reported (Conrad et al., 1978) that the mechanism responsible for the decreased ocular bioavailability of pilocarpine from instilled solutions of lower pH should include consideration of both an induced lacrimation effect and a pH-partition effect. In this regard, our experimental findings suggest that the more significant and, in fact, nearly totally determinant effect is the pH-partition effect and that its magnitude depends not necessarily on the pH of an instilled solution, but on the concentration of buffer contained in an instilled solution. The concentration of the buffer in an instilled solution upon mixing of precorneal fluid and drug solution dictates the time course of pH, drug ionization and therefore drug absorption.

Hind and Goyan (1947) also included buffer concentration or buffer capacity (index) as an important factor in formulation of ophthalmic solutions containing ionizable drugs. However, the validity of their qualitative statement has apparently not previously been tested. No quantitative report has been made relative to the importance of buffer concentration on the efficiency of ocular drug absorption. As a result, ophthalmic solution formulation has possibly and seemingly been conducted without consideration being given to this potentially significant formulation variable.

Ophthalmic solutions of amine drugs that are of therapeutic significance in glaucoma, such as epinephrine, pilocarpine and others, require an acidic formulation environment for reasons including chemical stability and/or solubility. However, these physicochemical demands are met at the expense of absorption efficiency or bioavailability of these amines, because they are delivered in an essentially totally ionized state. A systematic and definitive study of the compromise in bioavailability that results from the topical ophthalmic delivery of amines in acidic solutions has not been reported. It is the purpose of our studies to quantitatively describe the effect of specific buffers and buffer concentration on the ocular bioavailability of topically administered amine drugs. The purpose of this initial report is to describe

specifically the effect of different concentrations of citrate buffer on the ocular absorption efficiency or ocular bioavailability of pilocarpine in the rabbit following topical instillation of solution dosage forms.

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 instillation solutions used were aqueous, isotonic, buffered 1.0% w/v pilocarpine nitrate solutions. They differed only in their citrate buffer concentrations 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 sodium citrate and citric acid and 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 required for each solution was calculated by the freezing point depression method using literature L_{iso} values for drug, buffer components and sodium chloride (see Table 1 for the composition of the solutions). Prior to addition of water to final volume, adjustment was made to a pH of 4.75 by the addition of concentrated hydrochloric acid or sodium hydroxide. 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 4.1 ± 0.2 kg with a range of 3.9–4.5 kg. No particular animal pretreatment procedures regarding water, diet or environment were followed.

TABLE 1

COMPOSITION, CALCULATED BUFFER CAPACITY (β_{calc}) AND EXPERIMENTALLY MEASURED BUFFER CAPACITY (β_{expt}) FOR THE BUFFERED OPHTHALMIC SOLUTIONS OF PILOCARPINE NITRATE TOPICALLY ADMINISTERED TO THE RABBITS

Solution	I	II	III	IV
Total molar citrate concentration	0	0.055	0.075	0.110
Sodium chloride contained (w/v%)	0.72	0.36	0.17	0
β_{calc}	0.0006	0.0350	0.0475	0.0693
β_{expt} ($\pm$S.D.)	0.001	0.031 ± 0.004	0.049 ± 0.001	0.070 ± 0.002

Each solution contained 1.0% w/v of pilocarpine nitrate. Each solution was formulated at a pH of 4.75.

The miosis–time profiles were obtained as previously described (Mikkelson et al., 1973). Pupillary diameter meaurements were made using a cathetometer (Eberbach, Ann Arbor, MI) with an accuracy of ±0.1 mm. All pupillary diameter measurements were made by the same individual. The test animals were not anesthetized; therefore, normal instilled fluid and lacrimal fluid dynamics were unaltered or uninterrupted. The miosis studies were conducted in an isolated and constant environment to minimize auditory and visual stimuli, without outside interruption. An instillation volume of 25.0 μl was used in all experiments. A minimum of one week washout time between experiments in a single animal (either eye) was taken, because it was established that this period of time ensured the absence of a carry-over effect or contribution to the observed pharmacological response. The baseline, normal or pretreatment pupillary diameters were 6.9 ± 0.4 mm with a range of 6.5–7.3 mm.

Buffer capacity (index) measurement

A 0.5-μl volume of a standardized 0.1 N aqueous sodium hydroxide solution was added to 5.0-ml volumes of each buffered pilocarpine nitrate solution used in the miosis–time profile studies, and the resulting solution pH was recorded. The pH change affected by the addition of hydroxide was determined by subtracting the initial pH, which was 4.75, from the measured pH. The experimentally measured buffer capacities, β_{expt}, were calculated by dividing the equivalents of base added per liter of buffer solution (5.0×10^{-5} equivalents per 5.0 ml or 1.0×10^{-2} equivalents per liter) by the observed pH change. All pH measuremens were made following calibration of the system (Orion, Model 801, Ionalizer and SensoreX, Model 5200C, Combination pH/Reference Electrode) against a certified buffer (Fisher Scientific).

Buffer capacity calculation

Buffer capacity or index values were calculated, β_{calc}, as described by Butler (1964). The formulations included in this study contain a monofunctional acidic drug (pilocarpine nitrate) and a polyfunctional buffer system (citrate), each of which contributes to the buffer capacities of the formulations. For solutions of mixtures of monofunctional acids and bases or systems of polyfunctional acids and bases, where the successive pK_a values differ by greater than 1.3 and the system is not buffered at either extreme of the pH scale, the following equation (Butler, 1964), which is an expression of the Van Slyke equation, estimates buffer capacity within ±5.0%:

$$\beta_{calc} = \Sigma_i \, 2.303 \frac{K_{a,i}[H^+]C_i}{(K_{a,i} + [H^+])^2} \tag{1}$$

where the subscript, i, represents each component or ionization contributing to the total buffer capacity, C is the molar concentration of an individual component, K_a is the acid dissociation constant of an individual component, and $[H^+]$ is the proton or hydronium ion molar concentration. Eqn. 1 yields:

$$\beta_{calc} = \Sigma_i \, 2.303 \, f_{u,i} \times f_{d,i} \times C_i \tag{2}$$

where f_u and f_d are, respectively, the fraction undissociated and fraction dissociated of an individual component or conjugate acid-base pair, with $f_u = [H^+]/(K_a + [H^+])$ and $f_d = K_a/(K_a + [H^+])$.

Results and discussions

The instilled or experimental drug solutions were prepared using citrate buffer systems, because commercially available 1.0% pilocarpine nitrate or pilocarpine hydrochloride solutions commonly contain a citrate buffer, either alone or in combination with another buffer, such as carbonate, borate or acetate. The composition of the experimental solutions is presented in Table 1. 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. The pH of 4.75 represents a point of essentially maximum buffer capacity for the system with respect to the second acid dissociation constant for citric acid ($pK_{a,2} = 4.74$).

The buffer capacity for the experimental solutions was calculated, β_{calc}, using Eqn. 2, which is a form of the Van Slyke equation. The values of β_{calc} are given in Table 1. The species or acid-base pairs that were considered as contributing to the buffer capacity at pH of 4.75 and consequently included in the calculations were protonated pilocarpine ($pK_a = 6.88$). The second proton dissociation of citric acid ($pK_a = 4.74$) and the third proton dissociation of citric acid ($pK_a = 6.40$). It is

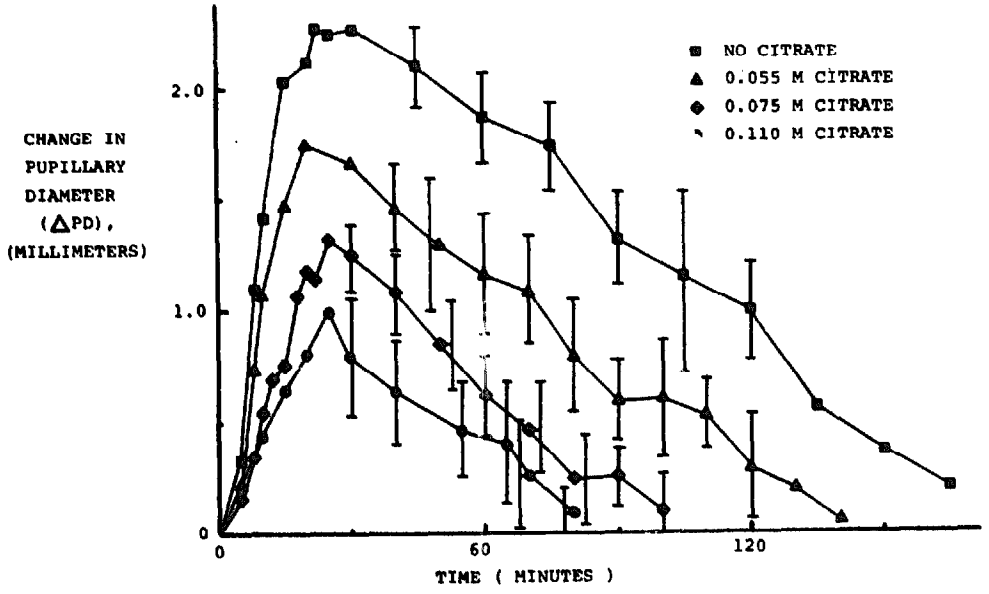

Fig. 1. Miosis–time profiles: plots of the average observed changes in pupillary diameter (ΔPD) as a function of time following the instillation of 25.0 μl of the isotonic 1.0% pilocarpine nitrate solutions, which contained the different concentrations of citrate buffer. The vertical lines through the data points are ± S.D. (data points with standard deviation lines omitted is for clarity of the figure).

apparent in the β_{calc} values and by Eqn. 2 that β_{calc} depends directly on the buffer concentration. The β_{calc} value of 0.0006 for the solution containing no citrate buffer (Solution I) is the contribution to the buffer capacity by the drug (1.0% w/v or 3.69×10^{-2} M) and consequently contributes, also, to the β_{calc} of Solution II (a 1.7% contribution), Solution III (a 1.3% contribution) and Solution IV (a 0.9% contribution). Table 1 also includes the experimentally determined buffer capacities, β_{expt}, which were obtained as previously described. They are seen to be in close agreement with the values calculated using Eqn. 2.

Following instillation of 25.0 μl doses of the 1.0% pilocarpine nitrate solutions of the different citrate buffer concentrations into the cul-de-sac of the experimental animals, the miotic response was recorded as a function of time by pupillary diameter measurement. The results of the in vivo studies are presented in Fig. 1, which is a plot of the average observed change in pupillary diameter at each buffer concentration as a function of time. Dramatic differences in pharmacological activity, as measured by the miotic response to the drug, are apparent. The general shapes of the profiles are similar, and the apparent slopes of the 4 profiles suggest similar elimination kinetics. However, the magnitude of the response to the drug and its duration are altered by and dependent upon the buffer concentration of the instilled solution. Significantly reduced miotic activity or relative pharmacological response can be observed to parallel an increase in buffer concentration. As a control, instillation of otherwise identical citrate buffer solutions containing no 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 is

TABLE 2

MAXIMUM OBSERVED PUPILLARY DIAMETER CHANGE (ΔPD_{max}), AREA UNDER THE MIOSIS–TIME PROFILE (AUC), AND THEIR RELATIVE VALUES FOR THE CITRATE BUFFERED PILOCARPINE SOLUTIONS ADMINISTERED TO THE RABBITS

Solution	I	II	III	IV
Total molar citrate concentration	0	0.055	0.075	0.110
n	6	7	6	8
ΔPD_{max} (mm)	2.45 ± 0.23	1.78 ± 0.23	1.35 ± 0.08	1.03 ± 0.25
ΔPD_{max}^{rel}	1.00	0.73	0.55	0.42
AUC (mm·min)	224 ± 22	124 ± 24	73 ± 14	40 ± 14
AUC^{rel}	1.00	0.55	0.33	0.18

The ΔPD_{max}^{rel} and AUC^{rel} values were obtained by dividing the individual solution values by those obtained for Solution I, which contained no citrate buffer. The value n is the number of experiments conducted on each solution.

TABLE 3

SIGNIFICANCE TESTS—ONE-WAY ANALYSIS OF VARIANCE OF THE AUC AND ΔPD_{max} DATA IN TABLE 2

Citrate buffer comparisons	P (AUC)	P (ΔPD_{max})
No buffer vs 0.055 M buffer	<0.01	<0.05
No buffer vs 0.075 M buffer	<0.01	<0.05
No buffer vs 0.110 M buffer	<0.01	<0.01
0.055 M buffer vs 0.075 M buffer	<0.05	<0.05
0.055 M buffer vs 0.110 M buffer	<0.01	<0.05
0.075 M buffer vs 0.110 M buffer	<0.05	<0.05

seen in the averaged data in Fig. 1 to occur from 20 to 30 min, and the area under the miosis–time profile, AUC, which was estimated using the trapezoidal rule, were determined for the individual experiments at each of the buffer concentrations. The averaged values of ΔPD_{max} and AUC at each buffer concentration are given in Table 2. Statistical significance tests conducted on the AUC and ΔPD_{max} data in Table 2, which was determined from the profiles in Fig. 1, is presented in Table 3. It can be seen that the differences observed in the AUC data are more significant than in the ΔPD_{max} data. In the AUC data for 6 possible comparisons, 4 are significantly different at the 99% confidence level and two at the 95% confidence level. Whereas, in the ΔPD_{max} data for the 6 possible comparisons, one is significantly different at the 99% confidence level and 5 at the 95% confidence level. The averaged ΔPD_{max} values reported in Table 2 do not coincide exactly with those in Fig. 1, because the observed maximum change in pupillary diameter in the individual experiments occurred at different times within the 20–30 min range. Relative values of the maximum observed pupillary diameter change, ΔPD^{rel}_{max}, and the relative area under the miosis–time profile, AUC^{rel}, are also reported in Table 2, where the reference values used in calculating the relative values were those obtained in the experiments using the solution containing no citrate buffer (Solution I). The solution containing a total citrate buffer concentration of 0.055 M (Solution II) resulted in a relative maximum pupillary diameter change, ΔPD^{rel}_{max}, of 0.73 and a relative miosis–time profile area, AUC^{rel}, of 0.55. The solution containing a total citrate buffer concentration of 0.075 M (Solution III) yielded a ΔPD^{rel}_{max} of 0.55 and an AUC^{rel} of 0.33. The solution containing a total citrate buffer concentration of 0.110 M (Solution IV) yielded a ΔPD^{rel}_{max} of 0.42 and an AUC^{rel} of 0.18.

The dependence of relative response on buffer concentration is given in Fig. 2, which is a plot of both average area under the miosis–time profiles and maximum observed change in pupillary diameter as a function of total concentration of citrate buffer contained in the 1.0% pilocarpine nitrate solutions. The lines drawn in Fig. 2 through the data points are trend lines. Both the AUC and the ΔPD_{max} appear to be reasonably linear with deviation occurring at high buffer concentration. As the citrate buffer concentration is increased, the rate of decline is seen to be greater in the AUC than in the ΔPD_{max}. The pilocarpine nitrate present in the formulations

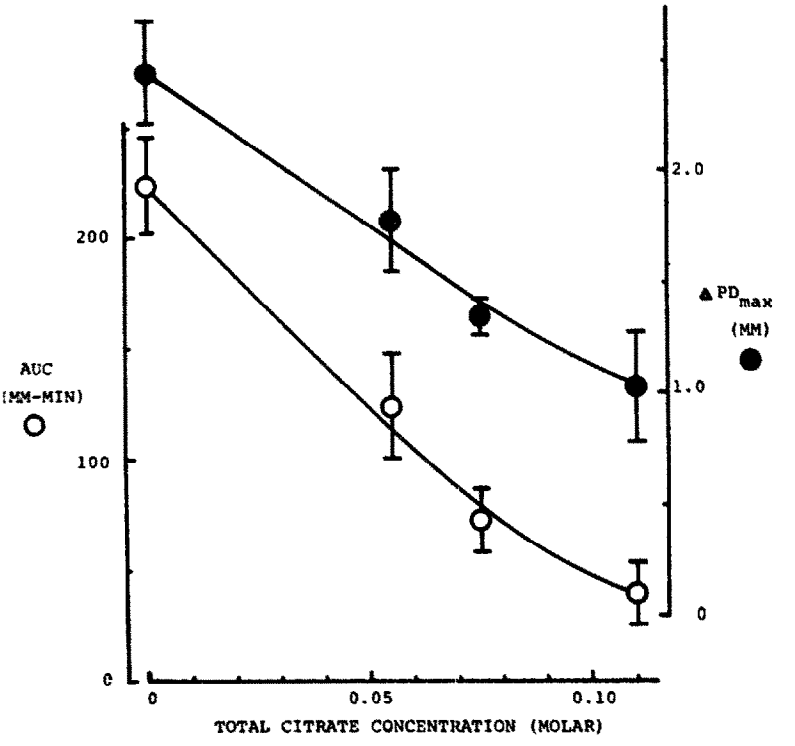

Fig. 2. Plots of the areas under the miosis–time profiles (AUC) in mm·min units and maximum observed pupillary diameter changes (ΔPD_{max}) in mm units as a function of total citrate buffer concentration contained in the formulations. The vertical lines are ± S.D. Symbols: ○, AUC; and ●, ΔPD_{max}.

also contributes to the buffer capacity, in addition to the citrate that is present. Therefore, when the same response parameters (AUC and ΔPD_{max}) are plotted as a function of the experimentally measured buffer capacities, β_{expt}, or the calculated buffer capacities, β_{calc}, a similar result is obtained.

A dramatic dependency of the efficiency of the ocular absorption of pilocarpine from instilled solution on the citrate concentration is evident in the two figures. The solutions were all prepared at an initial pH of 4.75, yet substantial reductions in miotic activity or bioavailability were affected by altering buffer concentration or buffer capacity. The data suggest that the actual pH at which an ophthalmic solution of pilocarpine is buffered is secondarily important in comparison to the actual concentration of buffer used.

A model inclusive of the various processes that constitute precorneal drug disposition was reported by Lee and Robinson (1979). The model included mathematical terms for previously identified processes, such as lacrimal fluid turnover, instilled fluid drainage and productive and non-productive drug absorption, in addition to a function for pH-induced lacrimation. These various processes are either productive or non-productive relative to the efficiency by which an instilled drug reaches an intended site of action, such as pilocarpine and the internal eye. Ocular bioavailability, as measured by aqueous humor drug levels, or response intensity (such as miosis or management of elevated intraocular pressure), is a net

result of the competing productive and non-productive precorneal disposition mechanisms. Sieg and Robinson (1976) discussed a particularly significant aspect or effect of the non-productive precorneal washout mechanisms (instilled fluid drainage and lacrimal fluid turnover) relative to absorption of drug into the inner eye. They pointed out that the volume-dependent drainage of an instilled solution is essentially completed within 5 min of dosing, having returned the precorneal volume to the physiological level of 7.5 μl within that time. This initial rapid loss of an instilled drug solution and subsequent dilution and loss due to normal tear turnover infers that a precorneal drug gradient, necessary and responsible for productive corneal absorption, is of short duration. In later reports, Sieg and Robinson (1977) and Conrad et al. (1978) identified and discussed an apparent pH-induced lacrimation effect that further contributes to the rapid precorneal non-productive drug disposition. Their data suggest that the precorneal or lacrimal drainage rate constant following 25.0 μl doses is approximately 1.2–1.8-fold more rapid for an instilled solution pH of 4.75 than pH of 7.4. Although, instilled solution pH was controlled in their studies, the buffer capacity was uncontrolled, because different buffer systems were employed in the solutions at different pHs. They concluded that observed differences in ocular bioavailability as a function of instilled solution pH were due predominantly to pH-induced lacrimation and secondarily due to a direct pH-partition effect. Our data suggest the opposite to be true in that pilocarpine solutions of an identical pH (4.75) resulted in reductions in bioavailability of greater than 5-fold as a function of buffer concentration, whereas significantly smaller changes in precorneal drainage rate constants as a function of pH have been reported (Sieg and Robinson, 1977; Conrad et al., 1978).

The hydrolytic degradation of pilocarpine has been reported (Chung et al., 1970) and summarized (Connors, 1979) wherein the pH of maximum stability has been predicted to be 5.1. Consequently, formulation of pilocarpine nitrate and hydrochloride ophthalmic solutions in the pH range of 4.0–5.5 is necessary for maximum or acceptable chemical stability. Hydrolytic degradation of pilocarpine yields the acidic product, pilocarpic acid. As a result, unbuffered or very weakly buffered pilocarpine salt solutions become more acidic as degradation occurs, which as a result, increases the rate of hydrolysis due to specific acid catalysis. To overcome this pH-sensitive formulation problem, ophthalmic solutions of pilocarpine are maintained within a stable pH range by the inclusion of suitable buffer systems. In the absence of any general catalytic effects due to the buffer, maintenance of a desired pH and hence greater pilocarpine stability is ensured as the concentration of the buffer system is increased. However, as our experimental results indicate, this may be at the expense of pharmacological activity or bioavailability. Therefore, an implication of this study is that formulation decisions, such as buffer selection (type and concentration) should be made both on a basis of chemical stability and bioavailability.

For an amine drug, such as pilocarpine, the pH-partition prediction is that absorption or membrane transport is increased as pH (or fraction unionized) is increased. Instillation of 25.0 μl of an ophthalmic solution buffered at an acidic pH, such as a pilocarpine solution, upon mixing with the 7.5 μl of lacrimal fluid results in an acidic precorneal pH that is quite removed from physiological pH. An amine

drug, such as pilocarpine, would, as a result, be in a highly ionized state, which is undesirable from the standpoint of achieving efficient corneal absorption or transport. The length of time over which corneal drug absorption occurs, which is 5 min in the rabbit, has been reported (Sieg and Robinson, 1976). This limited time is a result of precorneal drainage mechanisms such as instilled fluid drainage, lacrimal turnover and induced lacrimation. During this limited time course of absorption, the pH of the precorneal fluids (and as a result the ionization state of pilocarpine) is a function of instilled solution drainage and the dilution by and mixing of the instilled solution with the physiological fluids being produced. The lacrimal fluid enters the precorneal space at a rate that is a summation of the normal production rate and the induced rate. It is, therefore, quite reasonable to assume that the time course of the return of the precorneal pH to physiological pH is a function of the resistance of the precorneal fluids to pH change, which is directly dependent upon the buffer concentration of the instilled drug formulation. The rate of return of the precorneal pH to physiological pH during the first 5 min following dosing, which is the time when productive absorption occurs, is decreased as the buffer concentration in the drug formulation is increased. As a result, during this time course when productive absorption occurs, less of the drug is converted to its more absorbable unprotonated or free amine form at higher buffer concentrations. The overall effect is that the buffer concentration in an ophthalmic pilocarpine formulation dictates the post-instillation time course of pH, the time course of the ionization state of pilocarpine (change from protonated to unprotonated form), the efficiency of pilocarpine absorption and the observed pharmacological response to the drug.

The data reported herein demonstrate a dependence of the pharmacological activity of pilocarpine, delivered as a solution, on the concentration of buffer contained in the formulation. Substantial reductions in response (e.g., $AUC^{rel} = 0.18$ at 0.110 M citrate buffer) were observed as a function of increased buffer concentrations. In general terms, these observed results demonstrate that the concentration of a non-drug entity, such as a buffer system, has a significant determinant effect relative to the response obtained from a drug contained in a simple solution dosage form. With particular reference to the development of oral dosage forms, Kaplan (1973a and b) has stressed the importance of conducting bioavailability studies in conjunction with preformulation and product formulation for the purpose of optimizing all aspects of product quality. In view of the apparent complex nature of precorneal fluid dynamics following the instillation of a drug solution and the results presented in this report, the suggestions of Kaplan (1973a and b) should be re-emphasized to include application to ophthalmic dosage forms. The results of this study are significant 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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