Buffering in Human Tears: pH Responses to Acid and Base Challenge

Leo G. Carney, Thomas F. Mauger, and Richard M. Hill

The buffering capacity of tears collected from six young, healthy subjects was assessed using a microtitration technique. Each subject provided, on six separate occasions, about 100 μl of tears, collected in small amounts and with minimal mechanical stimulation over several hours. The pH of the total stirred pool of tears from each subject was determined at the outset. This pool of tears was then divided into two equal volume aliquots, the pH of each being determined following each titration step of one of them with acid, and of the other with base. In all, 28 titration steps across the acid-base spectrum were completed for each patient pool collected. A total of 1044 tear pH measurements were made, all being done in a closed, temperature stabilized (36°C) microelectrode chamber having an accuracy of within 0.04 pH units. For a comparative reference, an identical titration procedure was used on degassed, demineralized distilled water (348 pH determinations). Buffering capacity was found to show considerable intersubject variations, but in all cases the effect was more pronounced and more uniform following acid titration. Local zones of enhanced buffering across the pH spectrum could be identified, presumably reflecting the multiple buffering components (bicarbonate, protein and others) present in tear fluid. Invest Ophthalmol Vis Sci 30:747–754, 1989

The pH of tear fluid is just one of its many physicochemical properties that contribute to the health and function of the anterior ocular tissues. Its values in health, disease and after environmental stress have all been investigated. Although fluctuations in the normal pH of the precorneal tear fluid have been noted, this fluid usually maintains a relatively stable pH environment for the anterior ocular tissues. This stability, despite exposure to atmospheric changes, is most often attributed to the buffering provided, as in other extracellular fluids, by the bicarbonate system. Such tear fluid buffering may be an important component of the defense mechanisms for the outer tunics of the eye; although it could be overwhelmed by any substantial challenge, such as a chemical splash, it should serve to lessen such impact. Certainly, it has considerable relevance in determining the efficacy of topically applied drugs, through the influence of pH on the state of drug ionization.

Despite its clear significance, relatively little is known about the magnitude of tear fluid buffering, its variability in the population, or the underlying mechanisms. Tear buffering action has been assessed previously in an in vitro system, but only for a limited range of alkaline challenge solutions. An in vivo microelectrode system has also been used to investigate tear film shifts in response to buffer instillation, although the specificity of such an electrode system has been questioned. Certain effects on this physiological parameter of age, disease and other variables remain to be explored, in addition to those more fundamental characteristics listed above.

In this report, we show results of tear buffering using finely graded incremental acid and base challenges over an expanded pH spectrum.

Materials and Methods

The six subjects in the study had a mean age of 23 years, with a range of 22 to 27 years. All had good general and ocular health, and none were contact lens wearers. All agreed to participate after the nature of the experiment was explained to them; the procedures following were approved by the Institutional Review Board (Human Subjects Committee) of The Ohio State University.

On six separate occasions, each subject contributed a total of 100 to 120 μl of tears. The tears were collected with microcapillary tubes from the lower tear prism, with care being taken to avoid mechanical stimulation. Subjects were instructed to monitor tear flow rate by observing the rate of tear accumulation.
in the microcapillary tubes, and to cease collection if flow rate notably increased.

A period of several hours was needed for each subject to accumulate the required volume, with each subsequent sample being sealed and then refrigerated upon collection until testing. Any variation in the subject’s normal activities, diet or health were noted. At least 1 day was allowed to elapse before the next tear collection occasion. For each subject, their collected tears were pooled and mixed in a closed vessel with minimal air space. The initial pH of each of these was measured using a closed-chamber, temperature stabilized (36°C) microelectrode system. Tear samples were drawn into the microelectrode with a manually operated suction device, which also allowed the samples to be subsequently evacuated into the closed-vessel for progressive titration. The pooled tears were then divided into two 50 µl aliquots. One 50 µl aliquot was progressively titrated with a total of 21 µg equivalents of hydrochloric acid, pH measurements being made at the following incremental titration levels: 0.02, 0.05, 0.10, 0.20, 0.40, 0.60, 0.80, 1.00, 1.50, 2.00, 3.00, 5.00, 11.00, and 21.00 µg equivalents. The second 50 µl aliquot was progressively titrated with a total of 1.45 µg equivalents of sodium hydroxide, pH measurements being made at the following incremental titration levels: 0.001, 0.0025, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.085, 0.12, 0.19, 0.33, 0.76, and 1.45 µg equivalents. These acid and base challenge doses were established empirically in pilot trials so that similar volumes of each challenge solution were used, the total volume of added acid and base being 300 µl.

An identical sequence was carried out, on 12 separate occasions, using degassed demineralized distilled water to establish a comparative reference (348 pH determinations).

The accuracy and reliability of the pH measurement system was established by making a series of ten consecutive readings on buffer standards of different pH levels (2.00, 6.77, 7.10, 7.50, 7.90, 10.00) after conventional calibration procedures. No reading from any of these series was found to differ by as much as 0.04 of a pH unit from the nominal value of the standard.

**Results**

The tear pH responses to these incremental titrations were analyzed in comparison to the pH response of the reference solution (unbuffered distilled water). Any deviations of the tear response from the response of the reference solution thus indicate the enhanced resistance of the nonaqueous tear components to pH challenge by acid or base. Comparing the tear response with that of an unbuffered reference solution also eliminates any bias from asymmetrical challenge doses. For the unchallenged tears, the mean pH value for the six subjects was 7.50 ± 0.16 (SD).

In Figure 1, the mean responses of all six subjects determined on six separate occasions, and at the initial and subsequent 28 titration levels, together with

![Fig. 1. Buffering capacity of tears shown as a comparison of tear pH change to that of a water reference solution upon titration with hydrochloric acid or sodium hydroxide. The sequence of titration levels for both HCl and NaOH to produce these pH changes is listed, with each challenge dose being given in microgram equivalents. Direction of increasing base challenge or decreasing acid challenge is left to right on diagram and downward on scale. Diagonal is equal response line. Results are mean (A) and standard deviation (B) of measurements for all six subjects on six separate occasions.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933148/ on 09/23/2017)
the standard deviations about those means, are shown. The most substantial deviation from the diagonal equal response line and hence increased resistance to pH change is in the range from tear pH 7.0 to pH 7.7, when the comparison unbuffered water values range from about pH 3.5 to pH 8.0. Beyond this plateau in the direction of increasing acid challenge, the tear pH falls precipitously to that of the water reference; less prominent zones of resistance to acid challenge are also present. The resistance to pH change with increasing base challenge is less substantial and less uniform. The standard deviations indicate that greatest variability in response is encountered, as would be expected, in those zones of acidic challenge where the pH is changing rapidly.

The mean responses of each of these six individual subjects from their six trials is shown in Figure 2. Each subject is seen to be relatively consistent within the overall pattern of response described above. However, two-way analysis of variance shows that there are statistically significant intersubject variations (P < 0.001; Table 1).

An estimate of the total buffering capacity provided by tears can be obtained by integrating the area bounded by the individual response curve and the equal response diagonal. This was done for each subject and for each of the six tear collection occasions. The mean results for each subject in response to acid and base challenges are shown in Figure 3. The greater total resistance to pH change can be seen to reside in the acid challenge direction (paired t-test, P < 0.001), where also the intersubject variability is less.

A critical feature of these pH response curves sometimes masked in the composite graphs is the usual presence of several quite prominent subunits of resistance to pH change. While almost always discernible in individual trials, slight variations in their appearance between subjects and in repeated trials on any one subject can result in their diminished prominence in the composite graphs. Results from the six individual measurement trials, with these resistance points indicated, are shown for a representative subject (subject 1) in Figure 4. A high degree of repeatability is seen amongst this subject’s trials. Considered overall for all six subjects, there was no statistically significant variation among trials (ANOVA, F5,35 = 0.998, P = 0.437).

Discussion

This titration technique provides quantifiable assessment of the resistance of tear fluid to pH change. The buffering is more substantial in response to acid challenge than it is to challenge with base. The values found here are of similar but slightly lower magnitude than those found with the limited titrations reported earlier.7,8 Because of differing experimental conditions, direct comparison with the published values derived from rabbit tears11 is not possible, but variations may be expected because of the functional and biochemical differences in tears acquired from this source. Of considerable relevance is the finding that the tear buffering is apparently nonuniform across the pH spectrum. This may be interpreted as being indicative of multiple buffering systems within each tear sample, each becoming predominant at specific pH values.

The major buffering system present within the tears is usually assumed to be the bicarbonate system,12 although recent measurements of bicarbonate ion concentration in tears are of lower values than earlier estimates.7 The substantial buffering capacity indicated by the plateau in the responses in the tear pH range of 7.0 to 7.7 may be a reflection principally of this bicarbonate system. However, other buffering systems would be expected to contribute in tears just as in other biological fluids. The major nonbicarbonate buffer components would likely be the various protein fractions present in tears.

The buffer capacity of any protein is a composite effect of the actions of its various dissociable groups. A chemical buffer will normally be most effective if its pKa is close to the pH under challenge. In the case of proteins, the presence of numerous acidic and basic groups means that a single pKa cannot be assumed. Rather, each of these various groups is titrated when acid or base is added; the resulting titration curve reflects the many possible pKa values within a given protein molecule. A major contribution to total protein buffering is usually attributed to the imidazole group of histidine,13 which has a pKa value within the physiological pH range (pKa 6.4 to 7.0). This possibly contributes importantly to the plateau pH response found here for tears. Beyond the physiological range, other dissociable groups, with their pKa values spread across the pH spectrum (eg, arginine, pKa 11.9 to 13.3; aspartic acid, pKa 4.0), may contribute to the buffer action.13 These may explain the subtle but measurable discontinuities in pH response across the extensive pH challenge doses used here (eg, in Fig. 4). Although these groups may play a lesser role in buffering within the common physiological limits, the buffer action of any solution containing multiple buffer elements nevertheless does reflect the additive buffering contributions of each element. In the case of tear fluid, the presence of a unique pattern of protein constituents14 may be of significance in ultimately interpreting its overall pattern of pH response. While other buffering components,
Fig. 2. Results plotted, as in Figure 1, for the mean responses for each subject over six separate trials.
Table 1. Two-way analysis of variance of pH responses to acid or base challenge of tears from six subjects

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F ratio</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (S)</td>
<td>5</td>
<td>23.88</td>
<td>4.78</td>
<td>55.54</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Titration level (T)</td>
<td>28</td>
<td>8280.22</td>
<td>295.72</td>
<td>3439.30</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>S x T</td>
<td>140</td>
<td>31.12</td>
<td>0.22</td>
<td>2.59</td>
<td>Not</td>
</tr>
<tr>
<td>Error</td>
<td>870</td>
<td>74.81</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1043</td>
<td>8410.03</td>
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</tr>
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</table>

such as phosphate compounds, should also contribute to the total response, low concentrations may preclude any major buffering role.

The results clearly indicate then the presence of buffering constituents in human tears. A further way of quantifying this buffering as it changes across the spectrum of pH challenges is to find at each point the range of challenge levels over which no significant difference in the tear pH is found. Such a statistical test must of necessity include a dependence on the accuracy of the measurement procedure. We used Tukey's studentized range (HSD) test for multiple comparisons, using a significance level of $P < 0.05$, to illustrate this. The results for the composite of the six subjects are shown in Figure 5. As increasing acid challenges (from the untitrated position) are presented, a substantial range of challenges producing no statistical difference in pH exists at first, demonstrating the considerable tear buffering present. Further challenges in the acid direction and those in the base direction produce smaller ranges of pH responses that are statistically indistinguishable. Such a statistical test may have application in comparative studies of buffering stability.

This statistical approach to describing buffering capacity, illustrated in Figure 5, also adds support to our description above of the presence of discrete zones of buffering resistance. The most extensive range of statistically indistinguishable pH responses is centered around titration level 14, which for the composite data of these six subjects is at tear pH 7.4. Other zones of such buffering are centered around approximate titration levels of 3, 22 and 26. These zones approximate tear pH values of 2.1, 9.0 and 10.3, respectively, and together with the above 7.4 pH value, could be considered to represent the pK' values of human tears. These population values show reasonable qualitative consistency with those indicated in Figure 4 for zones of enhanced pH resistance in individual trials for one subject.

There are considerable intersubject variations in both the form of the response curves (Fig. 2) to these finely graded incremental challenges, and in the overall buffering capacity provided by the tears (Fig. 3). While there is some variability in the response to acid challenge (indicated by the large standard deviations in Fig. 1), this is principally a consequence of the precipitous change in pH which usually occurs with titration in this direction. Base challenge also produces substantial individual variations, noticeable both in the form of the response curves and the total tear buffering in that titration direction. In the absence of any rapid change in pH, this is more probably an accurate reflection of variations in individual chemistry.

There are important clinical implications of this resultant net tear buffering. It helps provide a stable pH environment for the anterior ocular tissues, despite the unique variations in the ambient environment that exists for this body fluid. Among those variations are those associated with prolonged eyelid closure during sleep. It may be relevant to the ocular response to chemical splash. The small tear volume means that total tear buffering capacity will be limited. Nevertheless, while tear wash-out effects and even corneal buffering may be predominant, tear buffering should contribute to the overall response to challenge. It most certainly is of relevance.

![Graph showing buffering capacity](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933148/)
Fig. 4. pH response curves from the six individual trials of one representative subject (Subject 1). Points of increased resistance to pH change are indicated by arrows.
Fig. 5. Results of Tukey's studentized range (HSD) test for the composite of six noncontact lens wearing subjects. For each titration level (abscissa, full circle), ranges of statistically indistinguishable ($P < 0.05$) pH responses for other challenge levels are given (ordinate, open circles). As shown in the inset, position 15 is for unchallenged tears; position 1 is for a titration level of 21.00 μg equivalents of HCl; position 29 is for a titration level of 1.45 μg equivalents of NaOH.

in understanding and manipulating drug absorption properties, through the influence of pH on the state of ionization of drugs that are weak acids or bases. Corneal permeability is less to ionized than to nonionized molecules. Homatropine, tropicamide and cyclopentolate are all examples of drugs which are mostly ionized at physiologic pH and would exhibit low accessibility. This pH stability is also essential for some functions of the tear fluid itself, for example through its effect on the antibacterial activity of lysozyme.

The role of tear fluid buffering in damping tear pH changes may also influence the success of contact lens wear. Tear fluid pH has been demonstrated to be important in protein adsorption onto hydrogel lenses; additionally, it influences the water content, and hence oxygen permeability, dimensions and fitting characteristics of some current hydrogel contact lenses. By diminishing the magnitude of tear fluid pH shifts, particularly during the closed eyelid phase of extended wear, the integrity of the contact lens, and hence ultimately the health of the cornea, are protected, at least in part. Contact lens wearing itself may possibly lead to altered tear buffering through altered tear chemistry. Indeed, the intersubject variability in tear buffering may be one element contributing to some contact lens failures, particularly in extended wear regimens.

In summary, we have quantified the buffering capacity of human tears, and have demonstrated the considerable intersubject variability in this tear characteristic. The greatest extent of this buffering is to an initial acid challenge, but local zones of enhanced buffering distributed across the pH spectrum were also identified.

Key words: tears, buffering capacity, pK', bicarbonate, proteins

References