Effect of nonisotonic solutions on tear film osmolality

Frank J. Holly and David W. Lamberts

We found that the original osmolality of the tear film is regained rapidly subsequent to the installation of hypotonic artificial tears or other nonisotonic solutions. The minor irritation due to nonisotonicity and the increased tear volume both contribute to the increased turnover rate which in turn results in the short half-life (measured in seconds) of these solutions in the eye. Despite the expected fluctuation in tear volume and probably in tear secretion rate during the experiment, the osmolality of the tears followed an exponential decay with relatively good correlation.

Key words: tear film osmolality, hypotonic eyedrops, hypertonic eyedrops, tear secretion rate, tear volume, tear turnover rate, dry eyes, keratoconjunctivitis sicca

There has been a great deal of recent interest in tear film osmolality and its possible role in dry eye syndromes. Although previous workers\textsuperscript{1,2} had not been successful in demonstrating significantly elevated osmolalities in dry eye patients, more recently investigators\textsuperscript{3-4} have claimed that such a condition exists and have implied that the elevated tear osmolality may be responsible—at least partially—for the epithelial damage that occurs in sicca syndromes. Gilbard et al.\textsuperscript{4} determined osmolarity (numerically similar to osmolality but its value is a function of temperature because volume is a function of temperature) of aqueous tear samples as small as a few tenths of a microliter in both keratoconjunctivitis sicca (KCS) patients and normals and found that the sicca patients had elevated tear osmolarities. The authors claimed that the diagnosis of KCS can be made at the 95% confidence level if the tear osmolarity is greater than 310 mOsm/L.

The same authors\textsuperscript{4} also determined the tear osmolarity of KCS patients 1 hr after the instillation of isotonic tear substitutes. At this time they found that the eyedrops had no effect on the elevated tear osmolarity level of these patients, leading them to suggest that KCS patients may benefit from the use of hypotonic tear substitutes.

Following the publication of this work,\textsuperscript{4} a low-viscosity ($\eta = 2$ cps) and hypotonic artificial tear, Hypotears (Cooper Laboratories, Inc., Mountain View, Calif.), was introduced to the market and is presently distributed in the United States. The formulation has a claimed osmolality of 210 mOsm/kg.

The lacrimal system of the eye is highly dynamic. The total tear volume in the normal eye was determined to be between 5 and 9 $\mu$L,\textsuperscript{5} although it was found that the palpebral fissure in the open eye is capable of containing 20 to 30 $\mu$L of fluid temporarily without spilling.\textsuperscript{6} The tear secretion rate can vary from the basal rate of about 1 $\mu$L/min in a
quiet, unanesthetized eye (0.3 μl/min in an anesthetized eye) to several hundred microliters per minute when reflex tear secretion occurs, depending on the degree of irritation. Thus, even in the unirritated eye, the tear turnover rate is about 17%/min. Since osmolality is a colligative property associated with the bulk of the aqueous tears (or other solutions) it is directly affected by the bulk tear flow.

Such considerations suggest that even if the whole tear volume were replaced with a hypotonic formulation, it would be completely exchanged in a few minutes with newly secreted tears, thus restoring the original osmolality. Hence, one could conclude that the reason isotonic collyria had no effect on the hyperosmolality of tears collected from KCS patients 1 hr after administration was the short lifetime of the eyedrops in the palpebral fissure rather than the isotonicity of the drops.

In the study reported here, the tear film osmolality in both eyes of six subjects was determined as a function of time subsequent to the instillation of Hypotears, a hypotonic tear substitute, and of Liquifilm (Allergan Pharmaceuticals, Hormiqueros, Puerto Rico), a supposedly isotonic tear substitute. In addition to the six normal subjects, one patient with a mild dry eye condition but normal tear osmolality and another with asymptomatic eyes but elevated tear osmolality were included in the study.

A simple physical model was constructed to simulate tear exchange in the eye in order to obtain a mathematical equation describing the variation of osmolality as a function of time under certain conditions. The quantitative aspects of the model were compared with our experimental results obtained by instilling various volumes of hypotonic and hypertonic sodium chloride solutions in the eyes of one normal subject and measuring the osmolality of the tear samples as a function of time.

Materials and methods

Collection of tear samples. Disposable Boralex (Rochester Scientific Corp., Rochester, N. Y.) glass micropipettes were used to collect exactly 5 μl tear samples from the inferior fornix of the subject. Depending on the size of the tear meniscus and the momentary tear secretion rate, the time required to collect the samples ranged from 3 to 20 sec. The drop size of Hypotears, applied directly to the eye from its commercial container, was 28 ± 4 μl. After the instillation of the artificial tear, the subject was not allowed to blink until the first sample was collected. Another drop of tear substitute was then instilled, the subject was instructed to blink once, and another tear sample was collected. Next, an additional tear-substitute drop was placed in the eye, and tear samples were taken at 0.5, 1, 2, 4, and 8 min after instillation. During this time no additional drops were given.
and the subjects were instructed to blink normally. Their blinking frequency ranged from about 10 to 20 blinks/min.

Several tear samples were collected from each individual prior to the instillation of the tear substitute in order to determine their respective normal tear osmolalities. The average of the osmolality values obtained with these tear samples ($c_0$), taken as 100%, was used to calculate the relative osmolalities of subsequent tear samples ($c/c_0$) taken after the instillation of the tear substitutes or the nonisotonic NaCl solutions.

The effect of the nonisotonic NaCl solutions on tear film osmolality was studied similarly, except that the size of the droplet was either 10 or 20 µl and was strictly controlled. Furthermore, for each aliquot of sodium chloride solution instilled, only one osmolality determination was made at a given time to avoid the possible error caused by the depletion of the tear volume in the palpebral fissure.

**Determination of tear osmolality.** The method used was based on the determination of the dew point over the tear sample enclosed in a small stainless steel chamber. The temperature depression required to reach the dew point is related to the vapor pressure of the tears, which in turn is related to osmolality. The instrument employed was a micro-osmometer Model 5100-B (Wescor, Inc., Logan, Utah). This instrument has an accuracy of ±2 mOsm/kg in the physiological range of 200 to 400 mOsm/kg. Careful calibration technique and frequent checking of the cleanliness of the microthermocouple in the sample chamber enabled us to maintain this accuracy throughout the project.

**Results**

**Effect of Hypotears and Liquifilm.** The effect on tear osmolality following the instillation of Hypotears was investigated thoroughly. Six normal subjects were chosen from volu-
Table II. Time dependence of tear osmolality in a normal subject with hyperosmotic tears after instillation of hypotonic tear substitutes

<table>
<thead>
<tr>
<th>Time elapsed (min after drop instillation)</th>
<th>Average relative osmolality of aqueous tears ± S.D. (%)</th>
<th>No. of eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypotears:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No drop</td>
<td>(345.0 ± 1 mOsm/kg) 100%</td>
<td>2</td>
</tr>
<tr>
<td>0.25 (after 1 blink)</td>
<td>81.8</td>
<td>1</td>
</tr>
<tr>
<td>0.5</td>
<td>90.6 ± 3.8</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>93.8 ± 2.3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>100.0</td>
<td>1</td>
</tr>
<tr>
<td>Liquifilm:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No drop</td>
<td>(334 mOsm/kg) 100%</td>
<td>1</td>
</tr>
<tr>
<td>0.1 (no blinking)</td>
<td>81.7</td>
<td>1</td>
</tr>
<tr>
<td>0.1 (after 1 blink)</td>
<td>88.9</td>
<td>1</td>
</tr>
<tr>
<td>0.5</td>
<td>91.0</td>
<td>1</td>
</tr>
<tr>
<td>1.0</td>
<td>98.2</td>
<td>1</td>
</tr>
</tbody>
</table>

teers. The group consisted of four men (23, 27, 39, and 44 years old) and two women (one 25 the other 45 years old). Prior to the measurements, the average tear osmolality for this group was 294 ± 8 mOsm/kg. The osmolality of the Hypotears used was also determined and was found to be 220 ± 4 mOsm/kg.

Each tear osmolality value measured was expressed as percent of his or her normal value obtained prior to the experiment. These relative tear osmolality values obtained as a function of time subsequent to the instillation of Hypotears (approximately 28 µl in volume) are shown in Fig. 1. The solid circles signify data obtained with tear samples taken from the eyes before the first blink occurred after the drop instillation. The tear osmolality values obtained after at least one completed blink are denoted with triangles. For each tear sample taken before 30 sec of elapsed time after drop instillation, a new droplet of Hypotears was instilled in the eye, and in this time interval the triangles indicate that one complete blink had taken place. Subsequent to these short time measurements, an additional drop of Hypotears was placed in the eye, and a tear sample was taken at 0.5, 1, 2, 4, and 8 min intervals. No additional eyedrops were instilled during this time period of 8 min. The subjects were instructed to blink normally.

The use of relative osmolality enabled us to average the results obtained for these 12 eyes. The relative osmolality of Hypotears with respect to the average of normal tear osmolality was 75%. After instillation and without a single blink, the eyedrop mixed with enough tears to increase the osmolality of the tear strip to 80%. After one complete blink and as soon as 10 sec after instillation, the tear osmolality was already 88% of normal. At 0.5 min after instillation, the tear osmolality became 94%, and at 1 min the tear osmolality was the same as normal within the standard deviation of the results.

Fig. 1 shows some tendency for the tears to become slightly hyperosmotic, then slightly hypo-osmotic before reaching their normal value. The magnitude of such changes, however, was smaller than the standard deviation of the data, so that the trend may be an artifact and in any case was insignificant.

Fig. 2 shows the data obtained by using Liquifilm. This artificial tear preparation had an average osmolality of 252 ± 7 mOsm/kg. Thus this tear substitute is also somewhat hypo-osmotic. The same group of subjects used in the Hypotears study was employed again on a different day. Their average tear osmolality immediately prior to the experiment was 296 ± 4 mOsm/kg. In terms of this osmolality taken as 100%, the relative osmolality of Liquifilm was 85%.

The same notation as before was used to display the results in Fig. 2. Even in the absence of blinking, the average tear osmolality decreased to only 91% upon the instillation of
Fig. 3. Variation of the difference between one and the relative tear osmolality with time subsequent to instillation of 10 or 20 μl of distilled water in the eye. The results are plotted on a semilogarithmic scale and straight lines shown were fitted by the least-square method.

Liquifilm. After one blink the tear osmolality became 95%. After 30 sec the tear osmolality was indistinguishable from normal.

Two additional subjects were studied to determine the variation of their tear osmolality after the instillation of hypo-osmotic tear substitutes. One subject was a 30-year-old woman with definite clinical indications of a mild dry eye condition. She had often experienced mild irritation in her eyes. Her conjunctival and corneal epithelia exhibited typical staining patterns with rose bengal and sodium fluorescein. Her Schirmer test results fluctuated. On the day of the experiment it was 25 mm (OD) and 14 mm (OS) per 5 min without anesthesia and 4 (OD) and 2 (OS) with topical anesthesia. However, her tear osmolality was 302 ± mOsm/kg and thus was within normal limits. The experiment performed on this subject was similar to those performed on normals. Hypotears was used as the test solution. The results are shown in Table I. After the elapse of 30 sec, her tear strip had already achieved its normal value.

The other subject was a 34-year-old man whose Schirmer test values were 27 mm (OD) and 13 mm (OS) per 5 min without anesthesia and 15 (OD) and 7 (OS) with topical anesthesia. No epithelial damage could be discerned by the usual staining techniques, and no subjective complaints were present to suggest even a marginal dry eye condition. His tear osmolality, however, was unusually high. On the day of testing, his tear osmolality was 345 mOsm/kg. The time dependence of the osmolality of his tear margins was determined after the instillation of Hypotears and after the instillation of Liquifilm (Table II). As the results show, even though the relative osmolality of Hypotears was 64% and that of Liquifilm was 75% with respect to his tears, his tear strip had become hyperosmotic again in 1 or 2 min after the instillation of the hypotonic tear substitutes.

Effect of distilled water and hyperosmotic salt solutions. In order to obtain more accurate data for kinetic analysis, additional measurements of tear film osmolality were conducted in one subject (a 44-year-old man) following the instillation of 10 or 20 μl of distilled water having zero osmolality and after the instillation of the same volumes of hypertonic aqueous sodium chloride solutions with osmolarities equal to 500 and 1000 mOsm/kg. For each osmolality determina-
Fig. 4. Variation of the difference between the relative tear osmolality and one with time subsequent to the instillation of 10 or 20 \( \mu l \) of hypertonic (500 mOsm/kg) sodium chloride solution. The treatment of the data is the same as in Fig. 3.

Fig. 5. Variation of the difference between the relative tear osmolality and one with time subsequent to the instillation of 10 or 20 \( \mu l \) of hypertonic (1000 mOsm/kg) sodium chloride solution. The treatment of the data is the same as in Figs. 3 and 4.

Upon examining the time variation of tear film osmolality induced by the instillation of nonisotonic drops, we found that the tendency to return to normal osmolality was so great that most of the tear samples had to be taken within the first minute subsequent to the instillation of the drops. This was especially true for cases when the volume instilled was only 10 \( \mu l \). Under such circumstances it
was imperative to collect 5 µl of tears in 1 or 2 sec in order to maintain reasonable accuracy in the time variable. Due to the temporarily increased tear volume, it was usually possible with skillful manipulation of the capillary micropipette to collect sufficient volumes of tear samples in such a short time.

The osmolality data obtained after the instillation of either 10 or 20 µl of distilled and deionized water into the cul-de-sac are shown in Fig. 3. In this figure, the logarithm of a quantity related to relative osmolality, 1 - c/c₀, is plotted as a function of time. The experimental results can be fitted well with a straight line, indicating that the tear turnover coefficient, k, remained reasonably constant during the time course of the experiment.

Fig. 4 contains results obtained when the osmolality of the instilled solution (10 or 20 µl) was 500 mOsm/kg (about 1.7 times that of normal). The results obtained with sodium chloride solution having 1000 mOsm/kg osmolality (about 3.3 times normal tear osmolality) are shown in Fig. 5. In these cases the time dependence of the logarithm of the quantity, c/c₀ - 1, is plotted and is shown to be approximately linear.

Discussion

As expected, our results show that the osmolality of the tear meniscus and thus the tear film in normals and in subjects with elevated tear osmolality can be lowered temporarily by the instillation of hypo-osmotic tear substitutes. However, the lowered tear osmolality increases rapidly with time. In a few seconds, even before the first blink occurs, the osmolality is already considerably higher than that of the solution instilled, mainly because of mixing with the tears in the palpebral fissure.

After the first blink, which assists in mixing and in eliminating some of the increased tear volume, the tear osmolality becomes even higher. In about 1 min the original tear osmolality is restored even if the instilled droplet initially had zero osmolality (deionized or distilled water).

Similarly, subsequent to the instillation of hyperosmotic solutions in the eye, the tear film osmolality soon returns to normal. All the results obtained with the various osmolalities and instilled volumes could be approximated with a straight line when the logarithm of (1 - c/c₀) [or (c/c₀ - 1) for hyperosmotic drops] is plotted vs. time. Thus the tear osmolality varies exponentially with time with an approximately constant tear turnover coefficient according to the following equation:

\[ c = c₀(1 + Ae^{-kt}) \]

where A and k are constant and A is <0 for hypo-osmotic solutions and >0 for hyperosmotic solutions. Other types of functions such as power function, logarithmic function, hyperbolic function, and linear function were also fitted to the experimental data and in all cases exhibited a poorer fit as indicated by the magnitude of the correlation coefficient.

Physical model of tear dynamics. It is often instructive to construct a simple physical model for a biological or other system and describe its functioning quantitatively while

### Table III. Measured and calculated parameters of tear kinetics subsequent to instillation of nonisotonic eye drops

<table>
<thead>
<tr>
<th>No. of exp.</th>
<th>Instilled drop</th>
<th>Kinetic parameters</th>
<th>Initial tear values</th>
<th>Half-life value (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c₁ (mOsm/kg)</td>
<td>V₁ (µl)</td>
<td>A</td>
<td>k (min⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>10</td>
<td>-0.709</td>
<td>2.81</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>20</td>
<td>-0.538</td>
<td>2.17</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>10</td>
<td>0.444</td>
<td>2.05</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>20</td>
<td>0.606</td>
<td>1.52</td>
</tr>
<tr>
<td>5</td>
<td>1,000</td>
<td>10</td>
<td>1.001</td>
<td>1.69</td>
</tr>
<tr>
<td>6</td>
<td>1,000</td>
<td>20</td>
<td>1.638</td>
<td>1.60</td>
</tr>
</tbody>
</table>
imposing fewer and fewer restrictions on the model.

For the lacrimal system we assumed that the palpebral fissure and fornices can be represented by a reservoir containing aqueous tears of volume V. The reservoir is supplied by the tear gland at a flow rate of $F_{in}$. In case of elevated osmolality, osmotically driven water flow through the conjunctiva would contribute to this "inflow." The reservoir has facilities for outflow at the puncta at the rate of $F_{out}$, which most likely depends on the magnitude of V. Any loss of fluid through the conjunctiva is included in this outflow rate. Water loss through evaporation, which has been estimated to have a rate of less than 0.1 µl/min, and its effect on tear osmolality have been neglected in this model.

It is further assumed that blinking succeeds in maintaining a uniform osmolality, c, throughout the reservoir. Thus the tears leaving the reservoir have osmolality c. The tears entering the reservoir have a tear osmolality, $c_0$, normal for the individual.

If the tear volume immediately prior to instillation of a droplet of volume $V_s$ and osmolality $c_s$ is $V_i$ with an osmolality $c_0$ after complete mixing, the initial tear osmolality of the mixture of tears and nonisotonic drop will be given as follows:

$$c_i = \frac{(c_0V_i + c_sV_s)}{(V_i + V_s)}$$  \hspace{1cm} (1)

provided that no spillage occurs. Equation 1 is identical to an equation of Mishima et al., except that in their paper the equation is solved for $V_i$, the initial tear volume.

The principle of the conservation of matter demands that the difference between the amounts of osmotic solutes entering and leaving the reservoir in a unit time equal the rate of change in the amount of osmotic solutes in the reservoir. The amount of osmotic solutes can be expressed as the product of the volume and concentration. Thus, generally, one can write

$$V \cdot \frac{dc}{dt} = c_0F_{in} - cF_{out}$$  \hspace{1cm} (2)

where V is the tear volume in the reservoir and F is the flow rate.

**Steady state.** In a true steady state, $F_{in} = F_{out}$ and both F and V are independent of time. Then separation of variables in equation 2 becomes possible, and integration yields the following equation for $c = c_i$ when $t = 0$:

$$c/c_0 = 1 + Ae^{-kt}$$  \hspace{1cm} (3)

where

$$A = c_i/c_0 - 1$$

and

$$k = F/V$$

**Quasi-steady state.** In general, subsequent to the instillation of the droplet, both the inflow and outflow rates and the tear volume will be a function of time. If we assume that even though the flow rates vary, at any given time the outflow rate will be approximately equal to the inflow rate, then we have a quasi-steady state, and the differential equation 2 has the following solution for $c = c_i$ when $t = 0$:

$$\ln[(c_0 - c)/(c_0 - c_0)] = \int_{0}^{t} (F/V) dt$$  \hspace{1cm} (4)
The integral of the right side can be solved when the time dependence of F and V are known.

One special case of this quasi-steady state occurs when the functions describing the time dependence of F and V are similar. It has been observed that the tear volume increases monotonically with tear flow rate under quasi-steady-state conditions.\(^5\) This is probably true, since the outflow rate is a direct function of the tear volume and the volume increase due to inflow rate increase will enhance the outflow rate until the quasi-steady state is re-established. In this special case, then, the ratio of F and V will be constant, and the solution of equation 4 will again be equation 3, with a constant tear turnover rate. We believe that the reason we observed a simple exponential dependence of tear osmolality with time was because the ratio of tear flow rate and tear volume remained approximately constant during the experiment.

Hence, from the semilogarithmic plots, parametric constants A and k can be obtained. From A, the initial tear osmolality subsequent to drop instillation, \(c_0\), can be calculated (cf. equation 3). Knowing \(c_0\), the initial tear volume prior to drop instillation can be obtained with the help of equation 1. These calculated values together with the parametric constants are shown in Table III. The osmolality change as a function of time for both distilled water and hypertonic salt solutions are exhibited in Fig. 6. These curves all represent the equation

\[
\frac{c}{c_0} = 1 + Ae^{-kt}
\]

and the respective parameters were obtained from the semilogarithmic plots of Figs. 3, 4, and 5 which also contain the experimental data points. The correlation coefficients listed in Table III are indicative of the degree of fit for the data shown in these figures.

Coefficient A is negative for hypo-osmotic solutions. It is positive for hyperosmotic solutions and can be either smaller or greater than 1. The initial tear volume obtained from A, \(V_0\), is in good agreement with values obtained by Mishima et al.,\(^5\) with fluorophotometry. The tear turnover coefficient, k, on the other hand, ranges from 150% to 280% \(\text{min}^{-1}\), which is even higher than the high initial tear turnover specific rate observed by Mishima et al.,\(^5\) during the first several minutes following instillation of a 1 \(\mu l\) drop of fluorescein, which was about 60% \(\text{min}^{-1}\). This is no doubt the result of the relatively large drop sizes instilled and the nonisotonocity of the solutions, both of which are expected to induce increased reflex tearing and increased drainage. In addition, hyperosmotic eyedrops could induce osmotic water flow through the conjunctiva, whereas hyposmotic eyedrops could result in increased conjunctival absorption of water.

In case of exponential time dependence, it is customary to define half-life (\(t_{1/2}\)) values. This is the time interval necessary for the osmolality to reach a value halfway between the initial and the final tear osmolality values. In our case

\[
t_{1/2} = \ln 2/k
\]

where

\[
c_{1/2} = (c_i + c_f)/2
\]

These half-life values in seconds are also shown in Table III. It is of interest to note that the half-life of the effect obtained with distilled water is much shorter than that obtained with the hyperosmotic solutions.

The results obtained in this study were not entirely unexpected. The lacrimal system is designed to flush out noxious chemicals and debris from the preocular surface. This study merely provides another testimony to the efficiency of the lacrimal system even in subjects with reduced tear secretion rates. From these findings it follows that the application of even maximally hypotonic, i.e., zero osmolality, solutions can have only a transient effect on the tear meniscus and tear film osmolality. Hence the application of such tear substitutes is of negligible value for normalizing abnormally high tear osmolality.

REFERENCES
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