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pH and Osmolality of Pre-corneal Tear Film and Commercially Available Artificial Tears

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Abstract

The pH and osmolality are the physico-chemical properties of tear film that play a strategic role in the health and function of the anterior ocular tissues. The aim of this study was to investigate the pH and osmolality of pre-corneal tear film and commercially available artificial tears.

Methods: A total of seventy-five subjects aged between 16 and 37 years with mean age of 21.20 ± 0.63 years were recruited for this study. A pack of Mandanol (37 vials) and 3 bottles each of the other 7 artificial tears were used for this study. The pH of the tear film and artificial tears was measured with pHep meter (Hanna Instruments Coy., UK), while the osmolality of the tear film and artificial tears was assessed with freezing point depression Osmometer (OSMOMAT 030, Gonotec Coy., Berlin, Germany).

Results: The mean pH and mean osmolality of the tear film were 7.42 ± 0.06 and 289.92 ± 4.36 mOsmol/kg, respectively. Optovisc® and Tear Naturale I® were hypotonic, Hypromellose® was isotonic and Tears Naturale II®, Aqua tears®, Microdrops®, Mandanol® and Ivymoicell® were hypertonic with osmolality between 309.50 and 363.25 mOsmol/kg. All artificial tears had pH between 7.03 and 7.58, except Mandanol® with pH of 7.90. There was no significant association between age and pH, osmolality, tear secretion. However, there was a significant association between age and tear stability. No association was found between pH and age; pH and osmolality; osmolality and tear stability, respectively.

Conclusion: Five hypertonic artificial tears are not recommended for use as they could induce ocular discomfort and stinging. Optovisc and Tears Naturale I lubricants are ideal for use as tear substitute for management of clinical and subjective dry eye.

Keywords: *pH; Osmolality; Tear Film Stability; Artificial Tears; Tear secretion; Osmometer*

Introduction

The optical integrity and normal function of the eye depend on an adequate supply of pre-ocular fluid covering its surface. Anatomically, the pre-ocular tear film is made up of the outer lipid (oily) layer secreted by the meibomian gland, the aqueous (watery) layer by the lacrimal gland and mucin layer by the goblet cells of the conjunctiva. Maintaining the structural integrity of these layers involves a complex interaction between the eye and the environment [1]. The major functions of the tear film include providing a smooth optical interface at the font surface of the eye by bridging minute irregularities in the surface epithelium, lubrication of the movement of the eyelids, protective function by removing foreign matter and the aqueous component contains lysozymes, lactoferrin, lipocalin which together with immunoglobulins (IgA and IgG), defensins and glycoproteins are responsible for the antimicrobial activity and defense. Desquamated corneal epithelial cells are also eliminated by the constant irrigation [2]. The tear film production, absorption and elimination constitute the dynamics of the tear film. The homeostatic balance of the tear film dynamics is responsible for the tear film stability which enables it to fulfill the vital roles. Any alteration in the production, retention and elimination will destabilize the tear film homeostasis and lead to tear instability and ocular surface changes [3]. The pH and osmolality (tonicity) are the most frequently encountered physico-chemical properties of the tear film and solutions (directly or indirectly applied on the eye), that contribute to the health and function of the anterior ocular tissues [4]. The normal pH of the pre-ocular tear film has been shown to demonstrate fluctuation in values. In spite of this, the fluid usually maintains a relatively stable pH environment for the anterior tissues [5]. Ocular discomfort and stinging have been associated with the use of solutions that differ from the pH of the pre-ocular tears [6]. The average pH tear film has been reported as 7.45 and the zone of ocular comfort as pH between 6.6 and 7.8. Ocular discomfort and stinging are associated with solutions that have pH outside the zone of comfort. The pH of artificial tears and contact lens multipurpose solutions should closely approximate that of the pre-ocular tear film in order to maintain the homeostasis of the tear film dynamics. Osmolality (tonicity or saltiness) on the other hand, is the total number of dissolved solute particles in one kilogramme of solution, without consideration of the nature of particles, that is, their shape, size, density, configuration or charge [7]. Tear Osmolality may be increased by any disorder that increases tear evaporation or decreases tear secretion including meibomian gland dysfunction, due to chronic meibomianitis and lacrimal gland disease, usually due to autoimmune mechanism [8]. Osmolality is an objective clinical measurement that provides insight into the balance of the complex tear film dynamics. The optical integrity of the cornea is significantly influenced by the tonicity of the tears. The tonicity of the human tears is subject to a dynamic change because of the evaporation process and the rate of tear flow. When evaporation is prevented, osmotic pressure of tears is equivalent to aqueous solution of 0.9% Sodium chloride (300 mOsmol/kg). When the aqueous component of tears decreases, the tears become markedly hypertonic (0.97% Sodium chloride solution or more) and corneal dehydration results [9]. Elevated tear film Osmolality negatively affects the ocular surface cells leading to pathological changes, such as a decrease in mucus-containing goblet cells, a decrease in intercellular connection, loss of microplicae and disruption of cell membranes [10,11,12]. Measurement of tear film osmolality has been suggested as a gold standard in the diagnosis of dry eyes as elevated tear film osmolality is considered as a core mechanism in symptoms and ocular surface damage in dry eye [13-16]. The use of hypotonic eye drops may provide symptomatic relief by reducing the hypertonicity of the tear film as demonstrated in the treatment of dry eye syndromes with moistening agents that act as tear-replacement therapy [9]. The therapeutic properties of artificial tears include tear film stabilization, ocular surface protection, reduced tear evaporation and enhanced lubrication and wound healing [17]. The comparison of the physico-chemical properties of the pre-corneal tear film and artificial tears would act as a guide in the selection of appropriate tear substitute for the management of clinical and subjective dry eye.

Aim of the Study

The aim of this study was to compare the pH and osmolality of commercially available artificial tears with human tear film.

Materials and Methods

The research design was an observational, prospective cross-sectional study carried out in Optometry clinic, Department of Optometry, University of Benin and Quality Research laboratory, Benin City, Nigeria.

The research protocol was approved by the Departmental Research and Ethics Committee in accordance with the tenets of the Declaration of Helsinki 2000. The tear film quality and quantity were assessed by measuring the tear flow rate with Schirmer I test strip and the lipid stability by non-invasive tear break-up time (NIBUT) [18]. The subjects were drawn from the University of Benin. Informed consent was obtained from each subject after the procedure and possible outcome were explained. Inclusion criteria were as follows, at least 18 years of age and no history of contact lens wear, systemic diseases associated with corneal pathology (rheumatoid arthritis), corneal pathology (e.g. infection, encroached pterygium, dystrophy, ectasias) of tear film abnormalities including symptoms of dry eye. All measurements were taken only in the right eye.

Collection of tear film sample

Disposable capillary tubes (Drummond Scientific Coy., Bromall, PA, USA) were used to collect tear samples from the mid-point of the lower tear meniscus of the right eye of each subject. The smallest sample quantity required for osmolality was 15 µl. To minimize the stimulation of reflex tearing, care was taken to ensure that the lid margin and corneal surface were not touched by the microtubes. Tear samples were collected in the laboratory under room temperature (25°C) and humidity. The required quantity of tear samples was transferred to the measuring vessels of the osmometer and 20 ml beaker for osmolality and pH measurement.

Measurement of the pH of tear sample and artificial tears

The pH of the aqueous solutions was measured with pHep meter (Hanna Instruments Coy., UK). The pH meter was first calibrated with a buffer solution of pH 7.0. After successfully calibrating the pH meter, the probe was rinsed with distilled water and was then wiped gently with the kimwipes to dry off any adhering solutions. 30 µl of tear sample was collected from the right eye of each subject using glass capillaries and transferred into a 20 ml beaker. The beaker was tilted while the pH meter probe was dipped into the tear sample to measure the pH. The measured pH value was automatically displayed on the screen. After measurement, the pH meter was removed, rinsed with distilled water and dried gently with the kimwipes. This procedure was repeated three times and the average value taken as the measured pH. For the artificial tears (AT), 10 ml of AT sample was transferred into a 20 ml beaker with a pipette. The pH meter was dipped into the AT samples to measure the pH. After about 45 seconds, the stabilized pH value displayed on the screen was recorded as the pH. The procedure was repeated four times for each AT sample and the average value taken as the measured pH. Table 1 shows the chemical components and manufacturers of commercially available artificial tears used in the study.

Table 1: Commercially available artificial tears, chemical components and manufacturers.

Artificial tears	Chemical components	Manufacturer
Ivy moicell®	Hydroxypropyl methylcellulose 0.7%, Benzalkonium Chloride 0.01%, sodium chloride, potassium chloride. Boric acid, Borax.	Ivee Aqua Epz, Kenya
Mandanol®	Hydroxypropyl methylcellulose 0.3%, sodium chloride Polyhexamethylene biguanide 0.0001%, potassium chloride, sodium perborate and Boric acid.	Sericon Pharm. PVT Ltd, India
Optovisc®	Hydroxypropyl methylcellulose 0.3%, Benzalkonium chloride 0.0002%.	Ashford Pharm. Lab, Phillipines
Hypromellose®	Hydroxypropyl methylcellulose 0.3%, sodium chloride Potassium chloride, Borax, Boric acid, Benzalkonium chloride sol. 0.01%, sodium hydroxide sol, Hcl, purified H_2O.	Sericon Pharm. PVT Ltd, India
Microdrops®	Sodium hyaluronate 0.2%, sodium chloride, disodium phosphate dehydrate, potassium dihydrogen phosphate.	Bohus Biotech, Sweden
Aqua tears®	Hydroxypropyl methylcellulose USP (0.7%), Borax BP (0.19%) Boric acid BP (0.2%), sodium chloride (0.45%), potassium chloride BP (0.01%), Benzalkonium chloride 0.01%.	CIPLA (protect), India
Tears Naturale I®	Hydroxypropyl methylcellulose 0.4%, Benzalkonium chloride 0.01%, disodium edetate 0.05%, dextran 70 (0.1%).	ALCON Eye Care Ltd, UK
Tears Naturale II®	Hydroxypropyl methylcellulose 0.3%, dextran 70 (0.1%), Polyquad 0.01%.	ALCON Eye Care Ltd, UK

Measurement of osmolality of aqueous solutions

The osmolality of pre-ocular tear film and artificial tears were measured with freezing point depression osmometer (OSMOMAT 030, Gonotec Coy., Berlin, Germany). The OSMOMAT 030 is a cryoscopic osmometer that measures the freezing point depression to determine the total osmolality of aqueous solutions. The freezing points of distilled water and a solution are measured and compared. While water has a freezing point of 0°C, a solution with a saline concentration of 1 Osmol/kg has a freezing point of – 1.858°C. The OSMOMAT 030 was calibrated with distilled water and a standard salt solution before measuring the total osmolality of sample solutions (pre-ocular tear film and artificial tears). The Gonotec calibration standard solution for OSMOMAT 030 was aqueous 0.9% sodium chloride (300 mOsmol/kg) [19]. A measuring vessel was filled with 15 μl of tear film or 50 μl of artificial tears (depending on which is being measured) and then pushed carefully onto the holder until the stop was reached. The thermistor probe (temperature sensor) was completely enveloped in the sample solution. The SAMPLE button was pushed. The vessel holder was pushed slowly but firmly down into the lower cooling system. The actual sample temperature was displayed in degrees Celsius on the display. Crystallization was initiated as soon as the temperature reached the super cooling temperature. This was achieved by inoculation of the sample with ice crystals from the stainless steel needle held directly above the sample vessel. The process of ice formation in the sample resulted in the release of heat which warmed up the sample. The temperature rose to the freezing point of the solution where a temperature plateau was reached which was monitored through the thermistor probe and interpreted by the measurement algorithm and electronically saved. The result was displayed in Osmol/kg. After a measurement was taken, the measuring vessel was manually moved to the upper position. The procedure was repeated four times and the average value was recorded as the measured osmolality of the human tear film or artificial tears.

Statistical analyses

SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) was employed for statistical analyses. Test for normality of distribution was performed with Kolmogorov-Smirnov Z statistic. Relationship or association between variables was determined with correlation and regression analyses. Statistical significance was reached when p-value was ≤ 0.05.

Results

A total of seventy-four (n = 74) subjects aged between 16 and 37 years comprising 33 males and 41 females with mean age of 21.2 ± 5.0 years were recruited for this study. Table 2 shows the descriptive statistics of the measured variables of the subjects recruited for the study.

Table 2: Descriptive statistics of measured variables.

Variable	N	Mean ± SD	Min	Max	Std Skewness	Std Kurtosis	95% CI
Age (years)	74	21.20 ± 5.40	16.0	17.0	1.00	0.02	19.97 - 22.43
Tear flow rate	74	21.55 ± 1.97	17.0	25.0	–0.41	–0.10	21.10 - 22.00
NIBUT	74	14.56 ± 1.914	11.06	18.6	–0.06	–0.78	14.11 - 15.01
Osmolality	74	289.92 ± 4.36	282.3	298.3	0.36	0.83	288.92 - 290.92
pH	74	7.42 ± 0.06	7.33	7.55	0.14	–0.96	7.22 - 7.62

SD = standard deviation; std skewness = standardized skewness; std kurtosis = standardized kurtosis;
Min = Minimum; Max = Maximum; CI = Confidence interval

The mean pH and Osmolality of the tear film of the subjects were 289.92 ± 4.36 (mOsmol/kg) and 7.42 ± 0.06, respectively.

The relationship between age and Tear flow rate (p = 0.30), Osmolality (p > 0.05), pH of tear film (p > 0.05) was not statistically significant. However, a statistically significant inverse relationship was found between NIBUT and age (r = – 0.24, p = 0.04). The linear regression equation of the association is represented by: NIBUT = 16.389 – 0.086Age. From this equation, the stability of the pre-corneal tear film decreases by 0.86s for every decade. The linear regression model is represented in figure 1.

The mean, standard deviation and confidence interval of the measured variables of the artificial tears used in this study are presented in table 3.

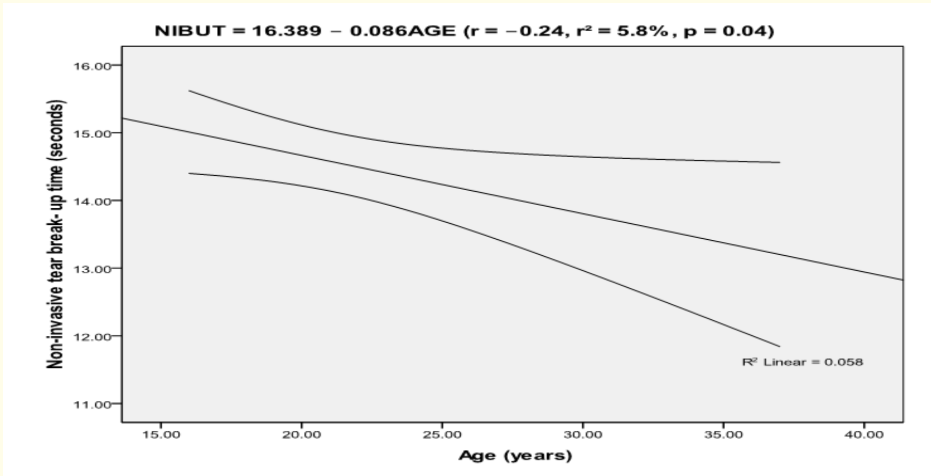

***Figure 1:** The trend line and 95% confidence interval of measured non-invasive tear break-up time versus Age.*

Table 3: Mean, standard deviation and confidence interval of the osmolality and pH of commercially available artificial tears.

Artificial tears	Mean Osmol ± SD (mOsmol/kg)	95% CI	Mean pH ± SD	95% CI
Aqua Tears®	333.75 ± 0.50	333.75 ± 0.49	7.58 ± 0.05	7.58 ± 0.06
Optovisc®	278.25 ± 0.50	278.25 ± 0.49	7.20 ± 0.08	7.20 ± 0.08
Mandanol®	363.25 ± 0.50	363.25 ± 0.49	7.90 ± 0.08	7.90 ± 0.08
Tears Naturale I®	284.25 ± 0.50	284.25 ± 0.49	7.03 ± 0.05	7.03 ± 0.06
Tear Naturale II®	309.50 ± 0.58	309.50 ± 0.57	7.45 ± 0.06	7.45 ± 0.06
Microdrops®	323.50 ± 0.58	323.50 ± 0.57	7.35 ± 0.06	7.35 ± 0.06
Hypromellose®	299.75 ± 0.50	299.75 ± 0.49	7.45 ± 0.06	7.45 ± 0.06
Ivy moicell®	331.25 ± 0.50	331.25 ± 0.49	7.35 ± 0.06	7.35 ± 0.06

SD = Standard deviation; CI=confidence interval; Osmol = Osmolality.

The association between tear film osmolality and NIBUT (p=0.52), tear flow rate (p = 0.42) and pH was not statistically significant.

The least osmolality of 278.25 ± 0.50 (mOsmol/kg) was reported for Optovisc artificial tears while the highest osmolality of 363.25 ± 0.50 (mOsmol/kg) for Mandanol. The highest pH of 7.90 ± 0.08 was reported for Mandanol while the least pH of 7.03 ± 0.05 for Tears Naturale I.

Discussion

The assessment of the tear film, as demonstrated by tear flow rate, inferred tear stability by non-invasive tear break-up time coupled with the pH and osmolality showed that the tear film of the subjects recruited in this study was normal. The tear film pH and osmolality are two physico-chemical properties that play important role in the assessment of the tear film dynamics. Osmolality is the concentration of all solutes in a given weight of water and is expressed as milliosmoles of solute per kilogramme of water (mOsmol/kg H_2O). In other words, it refers to the concentration of osmotically active particles in that solution. To ensure that tear film of the subjects was normal, the tear film dynamics was assessed by measuring the tear flow rate, the rate of evaporation, pH and osmolality (Table 2). The pH of the tear film is one of the physico-chemical properties that play an important role in the health and function of the anterior ocular tissues [4]. The mean pH value for the human tear samples in this study was 7.42 ± 0.1. Previous studies have reported the average pH of tear film as 7.45. This was similar to that reported by Janzsky and colleagues [20]. Fluctuations in the pH of the preocular tear fluid have been noted due to mucin secreted by the conjunctival goblet cells, secretion of the meibomian glands, transudation, conjunctival metabolism and CO_2 escape, which plays the most important role [20]. 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 [4]. Ocular discomfort (characterized by gritty sensation, burning sensation, mucous discharge, dryness, itching) and stinging have been associated with the use of solutions that differ from pH of tears [6]. The zone of ocular awareness or discomfort is outside the pH range of 6.6 to 7.8 [9]. The pH values of artificial tears used in this study ranged from 7.03 to 7.90. Seven out of the eight artificial tears had pH within the zone of ocular comfort of 6.6 to 7.8.

However, the relationship between the pH and the tear volume was not statistically significant. This result was in agreement with that reported by Fischer., *et al* [21]. They found no association between pH of the tear film and tear volume. Norn., *et al*. [22] also reported no significant relationship between pH and tear volume. The pH of tear film was not affected by age; and this may have been due to the age range adopted for this study, as the subjects were young adults (between 16 to 37 years). Cole and Jaros [23]reported a decrease in pH of tear film in subjects above 40 years.

Furthermore, the results from the current study also showed that the inverse relationship between the age and the tear film stability was statistically significant. From the linear regression model, a prediction of 0.86s reduction in tear film stability for every decade is made. This result was in agreement with that stated by Patel., *et al* [24]. They used the tearscope to evaluate the tear film stability in subjects between 18 and 89 years. They reported that tear film stability was lower in the aged eye. Similarly, Mathers., *et al*. [25] reported a decline in tear stability with aging. The major causes of the increased instability to aging have been hypothesized to be related to the decrease in quality of the "tear binding surface" and incomplete blink amplitude of the eyelids during blinks.

Studies have reported that younger people have lower lipid viscosity, higher lipid volume and lower rate of evaporation [26,27]. With increasing age the lipid viscosity increases, lipid volume decreases and rate of evaporation increases. A decrease in lipid-lipid interaction strength with increasing age could decrease the stability of tears. This is because the lipid-lipid interactions on the tear surface must be broken for the tear film to break up [28].

From this current study, no statistically significant association was found between age and tear osmolality ($p > 0.05$). This result was in agreement with that of Farris., *et al* [29]. They were one of the first groups to investigate the effects of age on tear film osmolality and their results proved that there was no significant effect of age on tear film osmolality. Craig and Tomlinson [30] designed a study to investigate the effects of age on tear film osmolality. They found no significant linear correlation between age and tear film osmolality.

Furthermore, the relationship between age and non-invasive tear break-up time was not statistically significant. This result was in agreement with that of Amaechi and Osunwoke [31]. They measured the tear break-up time in young adults using invasive and non-invasive methods and reported that tear break-up time was not affected by age. Similarly, the relationship between age and tear secretion was not statistically significant. This was consistent with the findings of Craig and colleagues [7], who reported that tear secretion remains unchanged with age. The relationship between stability and osmolality of the tear film was not significant. This was in agreement with Yeh., *et al* [32]. They compared the osmolality and stability of the tear film and found no clinically significant relationship between them.

The mean tear film osmolality value for normals obtained from this study was 289.92 ± 0.50 mOsmol/kg, which was slightly lower than the average osmolality values of 302.0 ± 2.9 mOsmol/kg, 304 mOsmol/kg and 303.7 ± 22.9 mOsmol/kg reported in previous studies [7,33,34]. The freezing point depression osmometry has been acclaimed the gold standard technique for assessing the osmolality of tears [10,35]. The tear film hyperosmolality has been considered as a differential feature of keratoconjunctivitis sicca or dry eye disease [8]. Considering the effects of hyperosmolality on the ocular surface, a good tear substitute for dry eye patients should be hypotonic [36]. Present study shows that the osmolality values of the artificial tears lie between 278.25 and 363.25 mOsmol/kg. Of the eight commercially available artificial tears evaluated, two (Optovisc and Tears Naturale I) were hypotonic compared to the mean osmolality of tear film (289.92 ± 0.50 mOsmol/kg), one (Hypromellose, 299.75 ± 0.50 mOsmol/kg) was isotonic, while the remaining five ATs (Aqua tears, Tears Naturale I, Microdrops, Mandanol and Ivymoicell) were hypertonic. Previous studies have shown that the osmolality values of most artificial tears evaluated were found to lie between 235.33 and 354.83 mOsmol/kg, with Thera® tears lying between 150 and 186.8 mOsmol/kg [37-40]. The moistening agents that act as tear replacement therapy which are hypotonic eye drops may provide symptomatic relief by reducing the hypertonicity of the tear film [9]. The hypertonicity and pH of solutions outside the zone of ocular comfort offset the homeostatic balance of the tear film resulting in tear film instability leading to ocular discomfort and stinging.

Conclusion

In conclusion, the average pH of the human tear film and all artificial tears except Mandanol was within the pH of the zone of ocular comfort. The five hypertonic artificial tears are not recommended for use as they could induce ocular discomfort and stinging. Two artificial tears, Optovisc and Tears Naturale I, were hypotonic and could be ideal for use as tear substitute for management of clinical and subjective dry eye. Tear film stability was found to be inversely correlated with age, while tear film osmolality, pH and tear secretion were not affected by age.

Conflict of Interest

The authors have no conflict of interest to declare.

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Volume 10 Issue 11 November 2019