Published in final edited form as:

Anal Biochem. 2020 November 01; 608: 113902. doi:10.1016/j.ab.2020.113902.

Fluorescent Contact Lens for Continuous Non-invasive Measurements of Sodium and Chloride Ion Concentrations in Tears

Ramachandram Badugu^a, Henryk Szmazinski^a, E. Albert Reece^{b,c,d}, Bennie H. Jeng^e, Joseph R. Lakowicz^{a,*}

^aCenter for Fluorescence Spectroscopy, Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, 725 West Lombard St., Baltimore, MD 21201, USA.

^bDepartment of Obstetrics, Gynecology and Reproductive Sciences, University of Maryland School of Medicine, 655 W. Baltimore Street, Baltimore, MD 21201, USA.

^cDepartment of Biochemistry and Molecular Biology, University of Maryland School of Medicine, 655 W. Baltimore Street, Baltimore, Md 21201, USA.

^dDepartment of Medicine, University of Maryland School of Medicine, 655 W. Baltimore Street, Baltimore, Md 21201, USA.

^eDepartment of Ophthalmology & Visual Sciences, University of Maryland School of Medicine, 419 W. Redwood Street, Baltimore, Md 21201, USA.

Abstract

Rapid and non-invasive measurement of hydration status is medically important because even mild levels of dehydration can have a significant impact on physical and cognitive performance. Despite the potential value of determining whole-body hydration based on the electrolytes found in tears, very few tests are available. An area of intense interest is the development of a contact lens which could measure ion concentrations in tears, specifically that of sodium (Na^+) and chloride (Cl^-) ions, the dominant electrolytes in blood plasma and tears. Here, we describe a method to make fluorescent contact lenses which allow determination of Na^+ and Cl^- ion concentrations in tears. Fluorophores known to be sensitive to Na^+ and Cl^- were derivatized to bind non-covalently to two commercially-available silicone hydrogel (SiHG) contact lenses—the Biofinity (Comfilcon A) or MyDay (Stenfilcon A) lenses. The sodium- and chloride-sensitive fluorophores displayed spectral

*Corresponding Author.

Author contributions

Ramachandram Badugu: Conceptualization, Investigation, Writing - Original Draft

Henryk Szmazinski: Investigation.

E. Albert Reece: Conceptualization, Writing - Review & Editing

Bennie H. Jeng: Writing - Review & Editing

Joseph R. Lakowicz: Conceptualization, Writing Original Draft, Review & Editing, Supervision.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Declaration of competing interest

Authors declare they have no competing financial interests.

changes in the physiological range for Na^+ and Cl^- ions in tears. The lenses for both Na^+ and Cl^- ions were completely reversible. The sodium responses were not sensitive to protein interference including human lysozyme, human serum albumin and mucin type 2. The chloride sensitivity was similar with both lenses, but the sodium-sensitive range was different in the Biofinity and MyDay lenses. We also fabricated a lens with both the Na^+ and Cl^- probes in a single MyDay lens resulting in a contact lens that independently measured Na^+ and Cl^- concentrations without physical separation of the fluorophores. Our findings indicated that a sodium and chloride-sensitive contact lens (NaCl-lens) could be used for rapid non-invasive detection of whole-body hydration, as well as associated diseases or other infections.

Graphical Abstract

Keywords

Fluorescence sensing; dry eye disease; hydration; contact lenses; tear composition; electrolytes in tears

Introduction

Rapid and non-invasive measurements of hydration status is medically important for all age groups, from infants to the elderly. Total body hydration is carefully controlled in a narrow range in humans [1–2]. Mild levels of dehydration can have a significant adverse impact on athletic [3–4] and cognitive performance [5]. In the elderly, the dehydration plays a role in dry eye disease (DED) [6] which affects 10 to 30% of the population and results in a significant decrease in quality-of-life [7–9]. Diagnosis of DED is complicated because of the many factors which contribute to its severity, including dehydration [6], Sjogren's syndrome [10], Meibomian gland dysfunction [11] and diabetes [12–13]. Measurement of the Na^+ and Cl^- ion concentrations in tears may aid in the diagnosis of these conditions.

DED, dehydration and diagnoses of other ocular pathologies can be improved by non-invasive measurements of the total electrolyte concentration in tears. Total tear milliosmolarity (TmOsm) is considered a reliable indicator for DED [14–15] and dehydration is related to the blood plasma osmolality (BPOsm) [16]. However,

determination of BPOsm requires collection of a fresh blood sample and a separate analysis, which is not practical during athletic events. TmOsm has been shown to follow BPOsm, and TmOsm is regarded as a promising non-invasive marker for rapid measurements of an individual's hydration status [17–19].

Very few tests are available for electrolytes in tears. This is because basal tears have a low volume, about 6 µl per eye [20–21]. Physical contact with the eye results in rapid production of additional tear fluid which changes the electrolyte concentration from the basal level tears [22]. The usual clinical assays for biomarkers and drugs cannot be used to detect ion concentrations, and there are no methods to measure all the individual electrolyte concentrations in tears. At present the dominant approach to measure tear electrolytes is based on the electrical conductivity of small samples collected by momentary contact of a detector device with the eye [23–25]. This approach provides a one-time reading and no information on the individual ion concentrations which contribute to the TmOsm. In 2016, a microfluidic system has been reported for measuring of Na⁺, K⁺, Ca²⁺ and pH [26] but this system does not provide continuous measurements and as of now has not been approved for clinical use.

In the present paper we describe ion-sensitive fluorophores (ISF) which are designed to bind to lenses and are sensitive to sodium and chloride concentrations. These are the dominant electrolytes in tears, and we anticipate measurements of both sodium and chloride, after suitable calibration and testing, will provide a clinically useful estimate of the body hydration and TmOsm of tears. In future work we anticipate synthesis and testing of ISF for the other electrolyte in tears.

Materials and Methods

The fluorescence probes, sodium green, (SG, tetramethylammonium salt, cell impermeant) 1-anilinonaphthalene-8-sulfonic acid (1,8-ANS), 6-propionyl-2-dimethylaminonaphthalene (Prodan) were purchased from Thermo Fisher Scientific – US. 6-methoxy quinoline, 1-bromooctadecane(OD-B), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), poly-L-lysine (PL) from Sigma-Aldrich (MW 70–150 kDa), and all other chemicals used for the present study were procured from Sigma-Aldrich. The Biofinity and MyDay silicone hydrogel (SiHG) lenses from Cooper Vision were used. Ultrapure water (with a resistivity of 18.2MΩ-cm) purified using a Millipore Milli-Q gradient system was used in the preparation of aqueous solutions.

The chloride-sensitive fluorophore used here has an octadecyl (OD) side chain, which binds hydrophobically to the non-polar regions of silicone hydrogel (SiHG) lenses, as was described in [27]. The sodium-sensitive fluorophore, sodium green, was linked to poly-L-lysine which binds to SiHG lenses. Two different sodium (Na-lens) and chloride sensitive (Cl-lens) lenses were made using different contact lens polymers (Biofinity and MyDay) which have very different chemical composition and demonstrates the general applicability of our approach.

Synthesis of the Sodium-sensitive and Chloride-Sensitive Fluorophores.

We synthesized a sodium-sensitive fluorophore by forming a covalent conjugate of Sodium Green (SG) with poly-L-lysine (PL). The conjugate SG-PL was prepared following activation of the free carboxyl groups on SG, and subsequent amide bond formation with PL (Figure 1). 0.15 ml of 0.01% PL from (MW 70–150 kDa) was added to the solution of 1 mg of SG (6.6×10^{-2} mmol) in 2 mL of dimethylformamide (DMF), which was activated with *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, 0.34 mg, 2.0×10^{-3} mmol) and *N*-hydroxysuccinimide (NHS, 0.2 mg, 2.0×10^{-3} mmol). The solution was stirred overnight at room temperature under an inert atmosphere. 0.015 mL aliquots of the as-prepared reaction mixture were diluted in 2 mL of water to bind the SG-PL probes to the contact lens. Fluorophore OD-MQB (Figure 1) was synthesized using 6-methoxyquinoline (MQ) and 1-bromooctadecane as described in references 28–30.

Labeling of Contact Lenses.

Lenses were labeled by incubating in 3 ml of a 1 μ M probe solution. Aqueous-methanol (50–50 v/v) solution of OD-MQB and aqueous solution of SG-PL were used for probe labeling. SG-PL and OD-MQB were found to bind rapidly to the Biofinity lenses, within one hour. Probe uptake was noticeably slower with the MyDay lens, which were left in the labeling solution for several days. The labeled lenses were extensively washed with deionized water to eliminate any loosely bound probe from the lenses before being used for the ion-responsive studies. The lenses were held diagonally in a 1 cm x 1 cm cuvette and covered with the appropriate buffer and salt concentrations. Unless stated otherwise all experiments were performed in 20 mM MOPS buffer, 8 mM potassium ion concentration, pH 7.2, at room temperature.

Fluorescence Spectroscopic Measurements.

Ion-sensitive fluorescence spectra (intensities) and intensity decays (time-resolved fluorescence) were measured using a Varian Eclipse 4 spectrofluorometer and FluoTime 300 instrument from PicoQuant (Berlin, Germany), respectively. The emission spectra and intensity data presented here in this manuscript are the average of three measurements. Intensity decays were analyzed with the EasyTau software. The 495 nm excitation source for SG-PL was from a Solea supercontinuum laser (PicoQuant, Germany) and the 375 nm excitation source for OD-MQB was from a laser diode (PDL 375, PicoQuant), both with a repetition rate of 40 MHz and a pulse width less than 100 ps. Some intensity and lifetime measurements for SG-PL were performed using Alba V (ISS, Urbana, IL) confocal fluorescence lifetime microscopy (FLIM) with the excitation source of a 473 nm laser diode with a repetition rate of 40 MHz and a pulse width near 250 ps. Intensity decays were measured at the emission maximum with the bandwidth of 10–20 nm analyzed in terms of the multi-exponential model,

$$I(t) = \sum \alpha_i \exp(-t/\tau_i) \quad (1)$$

where τ_i are the individual decay times and α_i the initial time-zero amplitudes. The average lifetimes can be represented in two ways [31]. The fractional intensity-weighted lifetime which is given by

$$\tau_f = \frac{\sum \alpha_i \tau_i^2}{\sum \alpha_i \tau_i} = \sum f_i \tau_i \quad (2)$$

where $\sum f_i = 1.0$. The product $f_i = \alpha_i \tau_i$ is proportional to the contribution of each component to the total steady state intensity. The term $\alpha_i \tau_i / \sum \alpha_i \tau_i$ is the fractional contribution of each lifetime component to the steady state emission at the measured wavelength. Equation 2 is appropriate for either a single fluorophore or mixture of fluorophores [31].

In the present report we use the amplitude-weighted lifetime given by

$$\tau_\alpha = \frac{\sum \alpha_i \tau_i}{\sum \alpha_i} \quad (3)$$

This expression can be used when the emission is from a single fluorophore with a changing quantum yield. In this case the normalized $\alpha_i / \sum \alpha_i$ value represents the molecular fraction of each decay component because the time-zero intensities are always proportional to the radiative decay rate Γ , which is the same for the same fluorophore. For a fluorophore with a single lifetime the quantum yield (Q) and lifetime (τ) are given by $Q = \Gamma / (k_{nr} + \Gamma)$ and $\tau = 1.0 / (k_{nr} + \Gamma)$ where k_r is the sum of the non-radiative decay rates. The usual cause for a change in intensity or lifetime is a change in the total value of k_{nr} , sometimes expressed as $\sum k_{nr}$. The value of Γ is determined by the extinction coefficient of the fluorophore, and is unchanged under most conditions [31]. If the multi-exponential decay is resolved the amplitude fractions of SG-PL represent the molecular fraction of free or Na^+ -bound forms.

Sodium Binding Constant.

The dissociation reaction for the sodium probe ($P = \text{SG-PL}$) complexed with Na^+ ions can be written as

where P is the free probe concentration with no bound sodium and $P - \text{Na}^+$ is the concentration of the sodium-bound probe. The dissociation constant K_D is given

$$K_D = \frac{[P][\text{Na}^+]}{[P - \text{Na}^+]} \quad (5)$$

The ratio of sodium-free and sodium-bound probe is given by

$$\frac{[P]}{[P - \text{Na}^+]} = \frac{K_D}{[\text{Na}^+]} \quad (6)$$

This expression shows the probe is 50% complexed with Na^+ when the concentration of Na^+ is equal to the K_D . We describe the binding affinity as the mid-point (MP) of the ion-dependent response where the spectral change is 50% complete. The mid-point value is typically a measure of K_D , which is used in many analytical applications. A larger value of K_D corresponds to a weaker binding affinity and a lower value of K_D indicates stronger

affinity for Na^+ ions. This approach yields the apparent value of K_D , and for sensing the mid-point is a useful parameter. If the quantum yield of the probe changes greatly upon ion binding, the apparent K_D is different from the actual K_D .

Quenching by Chloride.

The mechanism of chloride sensing is different from sodium, does not involve a binding reaction and there is no change in the OD-MQB emission spectrum in the presence of chloride. Chloride ions are spectroscopically silent and cannot be directly measured using UV or visible wavelengths. However, chloride is an efficient quencher of selected fluorophores, typically electron deficient aromatic molecules [32–33]. Collisional quenching occurs when excited OD-MQB has a diffusive collision with a chloride ion, which results in an immediate return of OD-MQB from excited state to the ground state. This effect which reduces the intensities (I) and lifetimes (τ) of OD-MQB, which are analyzed using the Stern-Volmer equations:

$$\frac{F_0}{F} = 1 + K[\text{Cl}^-] \quad (7)$$

$$\frac{\tau_0}{\tau} = 1 + K[\text{Cl}^-] \quad (8)$$

where F_0 and τ_0 are the intensities and lifetimes, respectively, in the absence of chloride. K is the Stern-Volmer quenching constant and the inverse of the chloride ion concentration needed for 50% quenching. For pure collisional quenching, which means no ground-state complex formation between OD-MQB and chloride, the values of F_0/F and τ_0/τ are typically the same [31]. For a fluorophore freely diffusing in solution, in the absence of a contact lens, the value of K and τ_0 can be used to calculate the bimolecular quenching constant. The value of K is the product of two parameters, the unquenched lifetime τ_0 and the bimolecular quenching constant k_q , that is $K = k_q\tau_0$. The value of k_q depends on the collision frequency of chloride with the fluorophore, and is therefore a measure of the chloride diffusion coefficient. In the lenses the chloride ion concentration may not be the same as in the bulk solution. We estimated the values of K and k_q by assuming the same concentration of chloride around the OD-MQB moiety in the lenses and in the bulk solution.

Results and Discussion

Selection of Contact Lenses.

At the present time, most contact lenses are made with soft polymers which absorb various amounts of water or tear fluid. Some lenses are still made with poly-hydroxyethylmethacrylate (HEMA), but most new lenses are based on SiHG. Because of the silicone regions and the interpenetrating polymer network (IPN) present in the SiHG lenses [34–35], they display high rates of transport of both ions and oxygen. While the SiHG lenses are satisfactory for many individuals, the polymer chemistry of lenses is evolving rapidly to further improve patient comfort and reduce adverse side effects. Some of the newer lenses undergo surface treatment to improve hydrophilicity [36], and it is not practical to test all

possible lenses. We selected two commercially available contact lenses which have high and low densities of SiHG, equivalent to low and high percentages of water. The selected lenses are the Biofinity and MyDay lenses, both from Cooper vision, which have very different chemical and physical properties, but have not been reported to have post-fabrication surface treatments. Biofinity lenses are based on the SiHG polymer Comfilcon A, which is approved for extended wear, up to 30 days. The oxygen permeability, expressed in Dk units, $[Dk = 10^{-11} (\text{cm}^3 \text{O}_2 \text{ cm})/(\text{ml sec Barrer})]$, which is the measure of oxygen passing through the contact lens with a certain pressure difference, in a given time [37–38]. The Dk value for the Biofinity lenses is 128 (Table 1). This value is higher than an equivalent thickness of water [39–41] which is near 80 Dk units. The high Dk values for the Biofinity lenses are due to silicone-rich regions which are known to be highly permeable to oxygen [42–43]. The Biofinity water content is 48% and the total silicone volume is thought to be about 30%. Even with this high silicone content the Biofinity lenses remain highly permeable to water and ions in tears. This permeability is due to the presence of a semi-interpenetrating polymer network (IPN) composed of continuous channels of pure water or tear fluid which extend from the front to the back surfaces of the lens (Figure 2). The channels are thought to be about 50 nm in diameter [44–45]. The macromolecular structural schematic in Figure 2 suggests the presence of low polarity and non-polar regions and the existence of interface regions.

The MyDay lenses, based on the silicone hydrogel Stenfilcon A, have become one of the most frequently prescribed lenses [46–47]. MyDay lenses are prescribed for one-day use and contain a very low silicone content of about 4.4% (Table 1). Even with this low silicone content the MyDay lenses still have high Dk values near 80. The high Dk value is claimed to be the result of “Smart Silicone Technology” which results in continuous silicone channels from the front to the back of the lenses. The IPN network in MyDay lenses is thought to be the opposite of the Biofinity lenses (Figure 2). The non-silicone region of the MyDay lenses may contain some non-silicone hydrogel. We expected the MyDay lenses to have interface regions for binding of hydrophobic ion sensitive fluorophores. Binding of SG-PL seemed possible because poly lysine is frequently used to coat hydrophobic surfaces [48–49] and hydrogels are typically negatively charged. These lenses are nearly identical in terms of patient comfort and corneal health [46–47].

Binding of Polarity Sensitive Probes to MyDay Lenses.

In polar solvents like water the emission of many fluorophores is shifted to longer wavelengths, the intensities can be much lower and the lifetimes much shorter. The probe 1-anilino-8-naphthalene sulfonic acid (1,8-ANS) displays a short wavelength emission maximum with high intensity and long lifetime in non-polar environments or in non-polar solvents like acetonitrile. However, it exhibits relatively weak and long wavelength emission with short fluorescence lifetime in polar or water-containing solvents (Figure 3A) [50–51]. 1,8-ANS bound rapidly to the MyDay lenses and displayed a short wavelength emission maximum and a long intensity decay time (Figure 3), even longer than that of the probe in acetonitrile (Table S1). The observed spectral properties of 1,8-ANS in lens are consistent with 1,8-ANS properties noticed in a non-polar environment [31]. We also used the probe 6-propionyl-2-dimethylaminonaphthalene (Prodan) which is also highly sensitive to local

polarity [52]. In contrast to 1,8-ANS, Prodan displays good emission in water but with a long emission maximum of 550 nm (Figure S1 and Table S2). In MyDay lenses the emission maximum is at a shorter wavelength than that in acetonitrile. These results indicated that the MyDay lenses contain non-polar regions, comparable to silicone.

Sodium Response of SG-PL in Lenses.

For clinical use the labeled lenses must display spectral changes at the physiological sodium concentrations present in tears, which are near 120 mM. The parent fluorophore, Sodium Green, has a sodium binding affinity near 5 mM [53–54]. At 100 mM Na^+ concentration the probe will be 95% in the sodium-bound form (Equation 6) and non-responsive to Na^+ at physiological sodium concentrations in tears. The Na^+ binding affinity of SG can be decreased by changing the di-azacrown to a mono-azacrown ether or a crown ether [55–58]. We reasoned that the sodium affinity of SG-PL may be lower in the lenses because covalent linkage to PL neutralizes the two carboxyl groups on the parent molecule SG (Figure 1) which may contribute to the sodium binding affinity. Additionally, the positive charges of the large poly lysine polymer may provide a repulsive effect on Na^+ and further decrease the affinity of SG-PL with Na^+ . Another possibility is the SG portion of SG-PL may become partially buried in the non-polar regions and have a decreased or eliminated sodium response.

The SG-PL emission spectra and intensity decays in MyDay and Biofinity lenses are shown in Figures 4 and S2, respectively. The intensities increase over 2-fold upon addition of NaCl to the surrounding solution. The intensity increased, the intensity decays became less rapid, the lifetimes became longer at higher Na^+ concentrations. The SG-PL intensity decays are strongly multi or non-exponential in the absence of Na^+ and appear to be less heterogeneous at high Na^+ concentrations. Results of the multi-exponential analyses are shown in Tables S3 and S4. Many fluorophores, including SG, especially when they are in non-homogeneous environments, display multi-exponential decays [59]. The amplitude-weighted lifetimes are adequate for sensing, and we make no attempt to interpret the separate decay components.

The sodium binding curves revealed different Na^+ affinities for SG-PL in Biofinity as compared to MyDay lenses (Figure 5). In the Biofinity lenses sodium binding is close to saturation at 120 mM Na^+ , but SG-PL in MyDay lenses displayed spectral changes up to 150 mM Na^+ , and are therefore responsive across the physiological Na^+ concentrations in tears. At present, we cannot explain the weaker Na^+ binding in MyDay as compared to Biofinity lenses. However, one possible reason could be that the sodium concentrations around the fluorophore are different in the two lenses studied. More specifically, the Na^+ concentrations near the fluorophore in Biofinity lenses could be similar to as in the bulk solution because the channels are thought to be pure water or tear fluid. In the MyDay lenses the silicone channels may be surrounded by other hydrogels which occupy about one-half of the volume around the fluorophore. These polymers could decrease the effective Na^+ concentration near the fluorophore. Overcoming this displacement effect requires higher bulk-phase Na^+ concentrations for binding. However, the overall sodium response of the lenses is satisfactory with the observed limit of detection and limit of quantification of

sodium ion with SG-PL labeled Biofinity lens are 0.2 and 0.7 mM, respectively. The corresponding values for SG-PL labeled MyDay lens are 0.3 mM and 0.8 mM respectively.

Reversibility and Protein Interference.

For clinical uses the spectral changes must be reversible for increases and decreases in Na^+ concentration. In Figure 6 we showed that the response of SG-PL was reversible, and the probe did not wash out of the MyDay lenses. The MyDay lenses were tested for reversibility by cycling between 0 and 320 mM NaCl. During each cycle, the lenses were rinsed several times in 3 ml of buffer then placed in buffers with and without NaCl. The intensity and lifetime changes were completely reversible (Figure 6). The reversible changes in fluorescence intensity (Figure 6 top) demonstrate that SG-PL is not washed out of the MyDay lenses, and the reversible lifetime response (bottom) shows the probe response is not affected by high or low sodium concentrations. A similar reversible response was noticed with SG-PL labeled Biofinity lenses (data not shown).

Another important consideration is the effects of proteins or other tear components binding to the lenses and changing the sodium response. Tears contain a large number of proteins and other biomolecules [60–61] and it was not practical to test all of these. Three proteins were selected which are known to be present in tears [62–63]; human tear lysozyme, human serum albumin and mucin type 2 (MUC2). The latter protein was commercially available in a partially water-soluble form. The response of SG-PL in MyDay lenses was not affected by the presence of 1 mg/ml of any of these three proteins (Figure 7). Also, no interference from these proteins was found for SG-PL in Biofinity lenses. Both lenses were exposed to these proteins for 2 hours. The results do not demonstrate that protein interference will not occur after longer contact with tear proteins. Because protein interference does not occur in 2 hours these lenses are already suitable for use in ophthalmology offices where the wearing and measured times will be shorter.

Chloride-Sensitive Contact Lenses.

Sodium sensing was achieved by direct binding of Na^+ to the fluorophore. Chloride sensing is accomplished by a different mechanism, collisional quenching. A large number of fluorophores are known which are quenched by chloride ions, and these are typically quinolinium or acridinium derivatives [64–65]. Collisional quenching occurs when a chloride-sensitive fluorophore undergoes diffusion-mediated contact with a chloride ion while the fluorophore is in the excited state. The amount of quenching is limited only by the highest possible concentrations of the quenchers, and not limited by a binding constant. The sensitivity to the quencher depends on the fluorescent lifetime in the absence of quenching (τ_0). A longer lifetime provides more time for a molecular collision and more quenching at a given chloride concentration (Equation 8). The unquenched lifetime τ_0 of the fluorophore must be long enough for quenching to occur. But if τ_0 is too long, the emission will be extensively quenched and too weak to measure.

Chloride-sensitive lenses were prepared using a quinolinium derivative OD-MQB (Figure 1). The quinolinium moiety in OD-MQB is ideal for chloride sensing in tears because of its unquenched lifetime near 18.5 ns (Table S5 and S6) which results in significant changes up

to chloride concentrations of 220 mM (Table S6), which is well above the physiological range. The Biofinity and MyDay lenses labeled with OD-MQB were both found to display significant quenching at physiological concentrations of chloride near 120 mM, which resulted in about 50% quenching in the center of the physiological range near 120 mM (Figures 8 and 9). Traditional intensities measurements (Figures 8 and 9, top) could be difficult if the patient's eye is moving or blinking. For this reason, we expect a clinically useful chloride-sensitive lens will contain a reference fluorophore which is not sensitive to chloride and displays emission in a different wavelength range [66]. A more direct measurement of the chloride concentration can be obtained from measurements of the fluorescence lifetime which can be independent of total intensity or intensity fluctuations. Chloride quenching was completely reversible in both Biofinity and MyDay lenses (Figures S3 and S4).

Analysis of quenching measurements is typically accomplished using the Stern-Volmer equation, which can be done using either intensities or lifetimes (Equations 7 and 8). This approach for OD-MQB in lenses is shown in both intensities and amplitude-weighted lifetimes (Figure 10). These plots show that both the intensities and lifetimes continue to change up to 200 mM chloride. This behavior is different from SG-PL where the binding site can be saturated at high Na^+ concentrations. In contrast quenching of OD-MQB, both intensity and lifetime, continue to decrease as the chloride concentration increased. The steady-state intensities and intensity decays can be used to calculate the bimolecular quenching constant k_q . This value can be interpreted by comparison with quenching of a fluorophore in solution without any steric barriers. For this measurement we used the water-soluble fluorophore 1-propenyl-6-methoxyquinolinium bromide (P-MQB) which is the same structure of OD-MQB but with a shorter, three carbon propenyl chain (structure not shown). The unquenched lifetime (τ_0) of P-MQB was 26 ns, and the Stern-Volmer constant K was 140 M^{-1} . From these values we calculated the k_q of $5.4 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ which is a typical value for efficient quenching [31]. Using the lifetime measurements for OD-MQB (Tables S5 and S6), we found values of 8.12 M^{-1} and 7.44 M^{-1} in the Biofinity and MyDay lenses, respectively. Using the unquenched lifetimes the respective values of k_q are 0.41×10^9 and $0.37 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$. These values show that quenching is reduced by about a factor of 10 for lens-bound fluorophores. The observed limit of detection and limit of quantification of chloride ion with OD-MQB labeled lens are 10 and 25 mM, respectively. The reduced quenching observed for OD-MQB in lens is a favorable result because the intensities and lifetimes are sensitive to chloride while the OD-MQB signal is not quenched too strongly. Importantly, the quenching constants are nearly the same in both lenses, which supports our conjecture that the sensing portion of OD-MQB is located in a similar aqueous phase. The 10-fold reduction in k_q from OD-MQB can be explained by chloride diffusion from only the aqueous side of the interface, by partial shielding of the quinolinium moiety in the silicon phase or the presence of other molecules located at the water-silicon interface.

Single Contact Lens Sensitive to Both Sodium and Chloride.

SG-PL and OD-MQB have widely spaced absorption and emission spectra (Figure 11). This result suggested the use of both probes in a single contact lens, to provide lens sensitive to both Na^+ and Cl^- (Na-Cl lens), without physical separation of the probes in the lens. The

absorption and emission spectra show that OD-MQB can be excited near 360 nm and with the emission observed at 454 nm. SG-PL will also absorb light near 360 nm but its emission at 550 nm will not overlap or interfere with measurement of the OD-MQB emission. SG-PL can be excited at 490 nm where OD-MQB does not absorb and will not interfere with the SG-PL measurements.

The sodium and chloride responses were measured for the doubly labeled MyDay lens. The response to chloride (Figure 12) was essentially identical to the response of the lens labeled only with OD-MQB (Table S7). This is a favorable but not an obvious result. It was possible for fluorescence resonance energy transfer to occur from OD-MQB to SG-PL in the small silicone volume of the MyDay lenses. Such an effect would decrease the unquenched lifetime of OD-MQB, change the calibration curve and decrease the sensitivity to chloride. Additionally, the sodium response of SG-PL remained the same in the presence of OD-MQB (Figure 13 and Table S8) showing that the presence of OD-MQB in the lens does not alter sodium response. These results demonstrate a contact lens to detect both sodium and chloride can be made without physical separation of the probes into different regions of the contact lens.

These our findings using sodium-, chloride- and sodium chloride-lenses inspired us to interpret the lens sensing response in terms of Boolean logic gates at molecular level. The logic gates have received huge attention due to their potential use in the development of molecular-scale electronic devices, which perform Boolean logic operations in response to the chemical inputs [67]. The sodium response with SG-PL labeled lens shows a Single-Input Single-Output (SISO), 'YES' logic gate behavior. Where we see the low- (or '0' state) and high emission intensity (or '1' state) of sodium lens in the absence and in the presence of sodium ion, respectively. In other words, only in the presence of sodium input, the fluorescence output state of the lens is '1'. Otherwise the fluorescence output is in '0' state. This behavior follows the Boolean logic expression $Y = A.1$, where A is the input state ('0' or '1') and Y is the output state ('0' or '1'). The corresponding truth table and schematic logic operation are shown in Figure 14. On the other hand, the chloride response from OD-MQB labeled lens follows a SISO, 'NOT' logic gate behavior with a Boolean expression of $Y = \bar{A}$, which means in the presence of chloride input, the fluorescence output state of the lens is '0', otherwise the fluorescence output state is in '1' (Figure 14). The observed sensing results with OD-MQB and SG-PL double probe labeled lens shows Single-Input Double-Output (SIDO) behavior with 375 nm excitation wavelength. As can be seen from Figure 12, the emission spectra show two band maxima, one at 450 nm and second one at 550 nm from OD-MQB and SG-PL, respectively. As discussed above, the interference on the emission spectra from one probe on the second probe is minimum, we can explain these results with Single-Input Double-Output Boolean logic. Here we considered NaCl as the single-input, and emission at 450 nm from OD-MQB (output 1) follows 'NOT' logic and the emission at 550 nm from SG-PL (output 2) shows 'YES' logic behavior (Figure 14). The sensing behavior for the double probe labeled lens with 495 excitation can simply attributed to SISO, 'YES' logic gate, similar to single SG-PL labeled lens (Figure 14). This logical behavior of the probes can potentially be useful for the device designing for the continuous monitoring of multiple tear analytes.

To the best of our knowledge this is the first report of a fluorescent contact lens which is directly sensitive to sodium concentration, and the first report of a lens sensitive to both sodium and chloride. Previous attempts to make fluorescent sensing contact lenses had limited success using hydrogel (HG) contact lenses because the probe response was low or the probe washed out of the lens [68]. However, the chemistry of contact lens polymers has changed from HEMA hydrogels to silicone hydrogels (SiHG) which contain low polarity regions, rich of silicone, and regions which are aqueous or tear fluid [34–35]. The interface between these regions provides a location to bind ISF with hydrophobic side chains and allow the ion-sensitive region to stay in the aqueous or tear phase. There is extensive literature on fluorophores which are sensitive to a variety of cations and anions [65–66]. These fluorophores are typically water soluble and respond to ions at intracellular concentrations. Many of the fluorophores which covalently bind to amino groups or other functional groups are not ion-sensitive fluorophores. One widely used reactive side chain on fluorophores are the acetoxymethyl (AM) esters. This functional group is cleaved by esterases for trapping in cells [69–70] but the AM esters essentially non-reactive with amines. Creation of a contact lens sensitive to all ions in tears will require synthesis of ISF with reactive groups on or near the fluorophore. With only a few exceptions the ISF are not available in forms which can be conjugated with biomolecules. Once these alternative ISF are synthesized it will be possible to fabricate contact lenses which are sensitive to any single electrolyte or all the electrolytes in tears, and the ion concentrations can be determined by remote detection of fluorescence. In contrast to other analyte-sensitive lenses, such as the Google lens and other electronic lenses [71–73] the proposed lenses will not contain any electronic components. The detection electronics will be in devices outside of the eye, and comparable to other instruments used in ophthalmology clinical care. The only presently available method to measure tear electrolytes is a measurement of the total milliosmolarity [23–25], which provides no information on the individual ion concentrations. This measurement requires contact with the eye to obtain a tear sample.

It can be difficult to imagine how the fluorescent lenses can be used in ophthalmology practice. However, the introduction of fluorescence sensing contact lenses is consistent with other emerging methods in ophthalmology. There has been a rapid introduction of new optical methods for in-vivo studies of tear films and corneal health. Some of these methods are automations of present measurements, such as tear break-up times (TBUT) using optical imaging and computer analysis in place of visual observation by ophthalmologists [74–75] or automated imaging of the Meibomian gland [76]. Other examples include the use of interferometry for measurements of both the tear films [77–78] and cornea imaging using optical coherence tomography [79–80]. Raman spectroscopy has been proposed to detect adenovirus in tears [81]. These methods are more complex than the proposed fluorescent contact lens. Also, when the mentioned methods were first introduced there was no evidence to correlate the measurements with disease states, but such evidence is being rapidly accumulated.

The situation is different for an ion-sensing contact lens. Multiple reports demonstrate that specific ocular changes occur with changes in specific ion concentrations in tears, such as correlation of magnesium concentrations in tears with glaucoma [82–83] and other

conditions. For these reasons we believe the ion-sensitive lenses will be rapidly adopted for research in ophthalmology and soon thereafter will be used during routine eye exams.

In summary, we described a method to make fluorescence sensitive SiHG contact lenses by placing ion sensitive probes. The probes sodium green or 6-methoxyquinoline were derivatized with hydrophobic or poly-lysine chains to bind silicone regions of lenses, while maintaining the ability to sense individual ionic species in aqueous regions of lens. The reversible response of contact lens to ions in physiological range is useful for continuous monitoring. To the best of our knowledge, this is the first report of a fluorescent contact lens sensitive to sodium concentration, and the first report of a lens sensitive to both sodium and chloride. Water-soluble probes are already known for other dominant electrolytes in tears. Our present approach can allow rational design of contact lenses for the total electrolyte profile of tears.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from the National Institutes of Health, S10OD19975 and S10RR026370, and for partial support from GM 107986, GM 129561 and GM.125986. The authors thank Dr. Amaya Mao for assistance with the figures. We also thank AAT Bioquest, Inc. for synthesis and donation of fluorophores not used in the current report, and iChekTear, Inc for valuable discussions. We also thank Dr. Julie Rosen for critical reading and editing.

References

1. Raman A, Schoeller DA, Subar AF, Troiano RP, Schatzkin A, Harris T, Bauer D, Bingham SA, Everhart JE, Newman AB, and Tylavsky FA (2004). Water turnover in 458 American adults 40–79 yr of age, *Am. J. Physiol. Renal Physiol*, 286:F394–F401. [PubMed: 14600032]
2. Casa DJ, Ganio MS, Lopez RM, McDermott BP, Armstrong LE, and Maresh CM (2008). Intravenous versus oral rehydration: physiological, performance, and legal considerations, *Curr. Sports Med. Rep.*, 7:S41–S49.
3. Walsh RM, Noakes TD, Hawley JA, and Dennis SC (1994). Impaired high-intensity cycling performance time at low levels of hydration, *Int. J. Sports Med*, 15:392–398. [PubMed: 8002117]
4. Casa DJ, Stearns RL, Lopez RM, Ganio MS, McDermott BP, Yeargin SW, Yamamoto LM, Mazerolle SM, Roti MW, Armstrong LE, and Maresh CM (2010). Influence of hydration on physiological function and performance during trail running in the heat, *J. Athl. Train*, 45:147–156. [PubMed: 20210618]
5. Ganio MS, Armstrong LE, Casa DJ, McDermott BP, Lee EC, Yamamoto LM, Marzano S, Lopez RM, Jimenez L, Bellego LL, Chevillotte E, and Lieberman HR (2011). Mild dehydration impairs cognitive performance and mood of men, *Br. J. Nutr.*, 106:1535–1543. [PubMed: 21736786]
6. Walsh NP, Fortes MB, Raymond-Barker P, Bishop C, Owen J, Tye E, Esmaeelpour M, Purslow C, and Elgbenzai S (2012). Is whole-body hydration an important consideration in dry eye? *Cornea*, 31:6622–6626.
7. Pflugfelder SC (2008). Prevalence, burden, and pharmacoeconomics of dry eye disease, *Am. J. Manag. Care*, 14:S102–S106. [PubMed: 18452369]
8. Miljanovic B, Dana R, Sullivan DA, and Schaumberg DA (2007). Impact of dry eye syndrome on vision related quality of life, *Am. J. Ophthalmol*, 143:409–415. [PubMed: 17317388]
9. Kastelan S, Tomic M, Salopek-Rabatic S, and Novak B (2013). Diagnostic procedures and management of dry eye, *BioMed. Res*, 2013:309723–1/6.

10. Phadatare SP, Momin M, Nighojkar P, Askarkar S, and Singh KK (2015). A comprehensive review on dry eye diseases: diagnosis, medical management, recent developments, and future challenges, *Adv. Pharmac.*, article ID 704946, pp 1–12.
11. Fron AJ, and Tiffany JM (2004). The contribution of Meibomian disease to dry eye, *The Ocular Surf*, The 2:149–164.
12. Kamel SS, Mohammed TH, El Zankalony YA, and Saad AH (2019). Prevalence of dry eye in diabetes, *J. Egypt. Ophthalmol*, 110:77–82.
13. Najafi L, Malek M, Ebrahim A, Aghili VR, Khamseh ME, Fallah AE, Tokhmehchi MRF, Jabarv M and Najafi B (2013). Dry eye and its correlation to diabetes microvascular complications in people with type 2 diabetes mellitus, *J. Diab. Compl*, 27:459–462.
14. Fuerst N, Langelier N, Massaro-Giordano M, Pistilli M, Stasi K, Burns C, Cardillo S, and Bunya VY (2014). Tear osmolarity and dry eye symptoms in diabetics, *Clin. Ophthalmol*, 8:507–515.
15. Sullivan BD, Whitmer D, Nichols KK, Tomlinson A, Foulks GN, Geerling G, Pepose JS, Kosheleff V, Porreco A, and Lemp MA (2010). An objective approach to dry eye disease severity, *Invest. Ophthalmol. Vis. Sci*, 51:6125–6130. [PubMed: 20631232]
16. Villiger M, Stoop R, Vetsch T, Hohenauer E, Pini M, Clarys P, Pereira F, and Clijsen R (2018). Evaluation and review of body fluids saliva, sweat and tear compared to biochemical hydration assessment markers within blood and urine, *Eur. J. Clin. Nutr*, 72:69–76. [PubMed: 28853743]
17. Ungaro CT, Reimel AJ, Nuccio RP, Barnes KA, Pahnke MD, and Baker LB (2015). Non-invasive estimation of hydration status changes through tear fluid osmolarity during exercise and post-exercise rehydration, *Eur. J. Appl. Physiol*, 115:1165–1175. [PubMed: 25576262]
18. Willshire C, Bron AJ, Gaffney EA And Pearce EI (2018). Basal tear osmolarity as a metric to estimate body hydration and dry eye severity, *Prog. Ret. Eye Res*, 64:56–64.
19. Fortes MB, Diment BC, Felice UD, Gunn AE, Kendall JL, Esmaelpour M, and Walsh NP (2011). Tear fluid osmolarity as a potential marker of hydration status, *Med. Sci. Sports Exer*, 1590–1597.
20. Von Thun Und Hohenstein-Biaul N, Funke S, and Grus FH (2013). Tears as a source of biomarkers for ocular and systemic diseases, *Exp. Eye Res*, 126–137. [PubMed: 23880526]
21. Scherz W, Doane MG, and Dohlman CH (1974). Tear volume in normal eyes and keratoconjunctivitis sicca, *Graefes Arch. Klin. Oph*, 141:141–150.
22. Fullard RJ, and Tucker DL (1991). Changes in human tear protein levels with progressively increasing stimulus, *Invest. Ophthalmol. Vis. Sci*, 32:2290–2301.
23. TearLab™ Osmolarity Test Card package insert. TearLab Corp (2008). 9.
24. Versura P, and Campos EC (2014). TearLab™ osmolarity system for diagnosing dry eye, *Exp. Rev. Molec. Diagn*, 13:119–129.
25. Szczesna-Lskander DH (2016). Measurement variability of the TertLab osmolarity system, *Con. Lens Ant. Eye*, 39:353–358.
26. Yetisen AK, Jiang N, Tamayol A, Ruiz-Esparza GU, Zhang YS, Medina-Pando S, Gupta A, Wolffsohn JS, Butt H, Khademhosseini A, and Yun S-H, (2017). Paper-based microfluidic system for tear electrolyte analysis, *Lab. On a Chip*, 17:1137–1148. [PubMed: 28207920]
27. Badugu R, Reece EA, and Lakowicz JR (2018). Glucose-sensitive silicone hydrogel contact lens towards tear glucose monitoring, *Biomed. Opt*, 23:1–5.
28. Badugu R, Jeng BH, Reece EA, and Lakowicz JR (2018). Contact lens to measure individual ion concentrations in tears and applications to dry eye disease, *Anal. Biochem*, 542:84–94. [PubMed: 29183834]
29. Ray K, Badugu R, and Lakowicz JR (2006). Langmuir-Blodgett monolayers of long-chain NBD derivatives on silver island films: well-organized probe layer for the metal-enhanced fluorescence studies, *J. Phys. Chem. B*, 110:13499–13507. [PubMed: 16821876]
30. Badugu R, Lakowicz JR, and Geddes CD (2005). Boronic acid fluorescent sensors for monosaccharide signaling based on the 6-methoxyquinolinium heterocyclic nucleus: progress toward noninvasive and continuous glucose monitoring, *Bioorg. Med. Chem*, 13:113–119. [PubMed: 15582456]
31. Lakowicz JR (2006). *Principles of Fluorescence Spectroscopy*, 3rd Ed. Springer, 954 pp.

32. Arosio D, and Ratto GM (2014). Twenty years of fluorescence imaging of intracellular chloride, *Front. Cell. Neurosci*, 8:1–12. [PubMed: 24478626]
33. Jayaraman S, and Verkman AS (2000). Quenching mechanism of quinolinium-type chloride-sensitive fluorescent indicators, *Biophys. Chem*, 85:49–57.
34. Kennedy JP, and Subramanyam U (2010). Polymer networks, producing crosslinking polyvinyl alcohol-polysiloxanes, and products and films made from networks, U.S. Pat. Appl. Publ (2010), US 20100305231, A1 20101202.
35. Chu S-H, and Cheng H-H. (2015). 3D network structured silicon containing prepolymer and method for its manufacture, U.S. Pat. Appl. Publ, US 20150252194 A1 20150910.
36. Subbaraman LN, Bayer S, Gepr S, Glasier MA, Lorentz H, Senchyna M, and Jones L (2006). Rewetting drops containing surface active agents improve the clinical performance of silicone hydrogel contact lenses, *Opto. Vis. Sci*, 85:143–151.
37. Pozuelo J, Compan V, Gonzalez-Meijome JM, Gonzalez M, and Molla S (2014). Oxygen and ionic transport in hydrogel and silicone-hydrogel contact lens materials: An experimental and theoretical study, *J. Mem. Sci*, 452:62–72.
38. Brennan N, and Morgan P (2009). Clinical highs and low of Dk/t, *Contact Lens Monthly*, 238:16–20.
39. Silicone hydrogels - What are they and how should they be used in everyday practice? Bausch & Lomb, CIBA Vision, *Contact Lens Monthly*, No. 5726, vol. 218.
40. Gromacki SJ (2013). Compliance with daily disposable contact lenses, *Contact Lens Spectrum/Special Edition*, p. 13 www.cispectrum.com
41. Morgan PB, Woods CA, Tranoudis IG, Helland M, Efron N, Grupcheva CN, Jones D, Tan K-O, Pesinova A, Santodomingo J, Malet F, Végh M, Erdinest N, Hreinsson HI, Chande P, Montani G, Itoi M, Chu BS, Bendoriene J Worp EVD, Awasthi S, Lam V, González-Méijome JM, Davila-Garcia E, Radu S, Belousov V, Johansson O, Silih MS Hsiao J and Nichols JJ (2012). International contact lens prescribing in 2011, *Contact Lens Spectrum*, 27:26–31.
42. Klimant I, and Wolfbeis OS (1995). Oxygen-sensitive luminescent materials based on silicone-soluble ruthenium diimine complexes, *Anal. Chem*, 67:3160–3166.
43. Sharma A, and Wolfbeis OS (1988). Fiber Optic Oxygen Sensor Based on Fluorescence Quenching and Energy Transfer, *Appl. Spectrosc*, 42:1009–1012.
44. Austin DTR, and Hills BP (2009). Two-dimensional NMR relaxation study of the pore structure in silicone hydrogel contact lenses, *Appl. Mang. Reson*, 35:581–591.
45. Guryca V, Hobzova R, Pradny M, Sirc J, and Michalek J (2007). Surface morphology of contact lenses probes with microscopy techniques, *Con. Lens Ant. Eye*, 30:215–222.
46. Osborn G, and Dumbleton K (2019). Eye care professionals' perceptions of the benefits of daily disposable silicone hydrogel contact lenses, *Con. Lens Ant. Eye*, 42:373–379.
47. Sapkota K, Franco S, and Lira M (2018). Daily versus monthly disposable contact lens: Which is better for ocular surface physiology and comfort? *Con. Lens Ant. Eye*, 41:253–257.
48. Aum H, Lvov YM, Mills DK, Jennings M, Alexander JS, and Jones SA (2003). Coating and selective deposition of nanofilm on silicone rubber for cell adhesion and growth, *Cell Biochem. Biophys*, 38:103–114. [PubMed: 12777710]
49. Harnett EM, Alderman J, and Wood T (2007). The surface energy of various biomaterials coated with adhesion molecules used in cell culture, *Colloids Surf Biointer*, 15:90–97.
50. Stryer L (1965). The interaction of a naphthalene dye with apomyoglobin and apohemoglobin: A fluorescent probe of non-polar binding sites, *J. Mol. Biol*, 13:482–495. [PubMed: 5867031]
51. Daniel E, and Weber G (1966). Cooperative effects in binding by bovine serum albumin. I: the binding of 1-anilino-8-naphthalenesulfonate. Fluorimetric titrations, *Coop. Effects Binding Albumin*, 5:1893–1900.
52. Weber G, and Farris FJ (1979). Synthesis and spectral properties of a hydrophobic fluorescent probe: 6-propionyl-2-(dimethylamino)-naphthalene, *Biochem*, 18:3075–3078. [PubMed: 465454]
53. Haugland RP (1996). *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed. Molecular Probes Inc., Eugene, OR, pp 503–584.

54. Szmazinski H, and Lakowicz JR (1997). Sodium green as a potential probe for intracellular sodium imaging based on fluorescence lifetime, *Anal. Biochem*, 250:131–138. [PubMed: 9245429]
55. Schreiner AE, and Rose CR (2012). Quantitative imaging of intracellular sodium, *Curr. Micro. Con. Adv. Sci. Tech*, 119–129.
56. Doussineau T, Schulz A, Lapresta-Fernandez S, Moro A, Korsten S, Trupp S, and Mohr GJ (2010). On the design of fluorescent ratiometric nanosensors, *Chem. Eur. J*, 16:10290–10299. [PubMed: 20665579]
57. Tusa JK and He H, (2005). Critical care analyzer with fluorescent optical chemosensors for blood analytes, *J. Mater. Chem*, 15, 2640–2647.
58. He H, Mortellaro MA, Leiner MJP, Young ST, Fraatz RJ, and Tusa JK, (2003), A fluorescent chemosensor for sodium based on photoinduced electron transfer, *Anal. Chem*, 75, 549–555 [PubMed: 12585483]
59. Berberan-Santos MN (2008). A luminescence decay function encompassing the stretched exponential and the compressed hyperbola, *Chem. Phys. Letts*, 460:146–150.
60. Shamsi FA, Chen Z, Liang J, Li K, Al-Rajbi AA, Chaudbry IA, Li M, and Wu K (2011). Analysis and comparison of proteomic profiles of tear fluid from human, cow, sheep and camel eyes, *Biochem. Mol. Biol*, 52:9156–9165.
61. Lorentz H, Heynen M, Kay LM, Dominici CY, Khan W, Ng WW, Jones L (2011). Contact lens physical properties and lipid deposition in a novel characterized artificial tear solution, *Mol. Vis*, 7:3392–3405.
62. Ohashi Y, Dogru M, and Tsubota K (2006). Laboratory findings in tear fluid analysis, *Clin. Chim. Acta*, 369:17–28. [PubMed: 16516878]
63. Gipson IK (2004). Distribution of mucins at the ocular surface, *Exp. Eye Res*, 78:379–388. [PubMed: 15106916]
64. Geddes CD, Apperson K, Karolin J, and Birch DJS (2001). Chloride-sensitive fluorescent indicators, *Anal. Biochem*, 293:60–66. [PubMed: 11373079]
65. Arosio D, and Ratto GM (2014). Twenty years of fluorescence imaging of intracellular chloride, *Front. Cell. Neurosci*, 8:1–2. [PubMed: 24478626]
66. Jayaraman S, Biwersi J, and Verkman AS (1999). Synthesis and characterization of dual-wavelength Cl⁻ sensitive fluorescent indicators for ratio imaging, *Am. J. Physiol*, 276:C747–C757. [PubMed: 10070003]
67. de Silva AP, Gunaratne HQN, and McCoy CP (1997). Molecular Photoionic AND Logic Gates with Bright Fluorescence and “Off-On” Digital Action, *J. Am. Chem. Soc*, 119:7891–7892.
68. Badugu R, Lakowicz JR, and Geddes CG, (2004) Noninvasive Continuous Monitoring of Physiological Glucose Using a Monosaccharide-Sensing Contact Lens, *Anal. Chem* 76, 3, 610–618 [PubMed: 14750854]
69. Hutton J, Potts B, and Southern FF (1979). Synthesis of 3,4-bi-acetoxymethyl-furan, *Synthetic Commun*, 9:789–797.
70. Slayman CL, Moussatos VV, and Webb w. W. (1994). Endosomal accumulation of pH indicator dyes delivered as acetoxymethyl esters, *J. Exp. Biol*, 196:419–438. [PubMed: 7823037]
71. Park J, Kim J, Kim S-Y, Cheong WH, Jang J, Park Y-G, Na K, Kim Y-T, Heo JH, Lee CY, Lee JH, Bien F, and Park J-U (2018). Soft, smart contact lenses with integrations of wireless circuits, glucose sensors, and displays, *Science Adv*, 4:eaap9841–1/11.
72. Chiou J-C, Hsu S-H, Huang Y-C, Yeh G-T, Dai K-S, and Kuei C-K (2017). Towards a fully integrated, wirelessly powered, and ordinarily equipped on-lens system for successive dry eye syndrome diagnosis, *Symposium VLSI Tech. Digest*, 1/2.
73. Lingley AR, Ali M, Liao Y, Mirjalili R, Klonner M, Sopanen M, Suihkonen S, Shen T, Otis BP, Lipsanen H, and Parviz BA (2011). A single-pixel wireless contact lens display, *J. Micromech. Microeng*, 21:125014–1/8.
74. Shimizu E, Ogawa Y, Yazu H, Aketa N, Yang F, Yamane M, Sato Y, Kawakami Y, and Tsubota K (2019). “Smart eye camera”: An innovative technique to evaluate tear film breakup time in a murine dry eye disease model, *PloS One*, 14:e0215130–1/13. [PubMed: 31071120]
75. King-Smith PE, Begley CG, and Braun RJ (2018). Mechanisms, imaging and structure of tear film breakup, *Ocular Sur*, 15:4–30.

76. Koproski R, Tian L, and Olczyk P (2017). A clinical utility assessment of the automatic measurement method of the quality of Meibomian glands, *BioMed Eng OnLine*, 1–13. [PubMed: 28086973]
77. King-Smith PE, Hinel EA, and Nichols JJ (2010). Application of a novel interferometric method to investigate the relation between lipid thickness and tear film thickening, *Cornea*, 51:2418–2423.
78. Nichols JJ, Mitchell GL, and King-Smith PE (2005). Thinning rate of the precorneal and prelens tear films, *Inves. Oph. Vis. Sci*, 46:2353–2361.
79. Chen Q, Wang J, Tao A, Shen M, Jiano S, and Lu F (2010). Ultrahigh-resolution measurement of optical coherence tomography of dynamic tear film changes on contact lenses, *Inv. Oph. Vis. Sci*, 51:1988–1993.
80. Del Aguila-Carrasco AJ, Ferrer-Blasco T, Garciz-Lazaro S, Esteve-Taboada JJ, and Montes-Mico M (2015). Assessment of corneal thickness and tear meniscus during contact lens wear, *Con. Lens Ant. Eye*, 38:185–193.
81. Choi S, Moon SW, Shin J-H, Park H-K, and Jin K-H (2014). Label-free biochemical analytic method for early detection of adenoviral conjunctivitis using human tear biofluids, *Anal. Chem*, 86:11083–11089.
82. Arutyunyan L, and Iomdina E (2013). Drug therapy of magnesium deficiency in patients with primary open angle glaucoma (POAG), *ARVO Ann. Meet*, 54.
83. Agarwal A, Lezhitsa L, and Agarwal P (2013). Pathogenetic role of magnesium deficiency in ophthalmic diseases, *Biometals*, 27:5–18.

Highlights:

- A method to make fluorescent silicone hydrogel contact lens to determine Na^+ and Cl^- ion concentrations in tears was described.
- The sodium- and chloride-sensitive fluorescence contact lenses displayed spectral changes in the tear physiological range, suitable for non-invasive continuous monitoring of ions in tear.
- A single lens with both the Na^+ and Cl^- probes shows the independent response to respective ions.
- This is the first report of a fluorescent contact lens to estimate sodium concentration, and the first report of a single lens sensitive to both sodium and chloride.

Figure 1. Chemical structures of sodium- (SG-PL) and chloride-sensing (OD-MQB) probes. Also shown are the polarity-sensing probes 1,8-ANS and Prodan.

Figure 2.

Semi-interpenetrating polymer networks for contact lens. Light blue area indicates silicone regions and light pink area indicates the water regions or tear fluid channels. The green dots show the locations of the ionic species in the lenses. The sodium- and chloride-sensing fluorophores bind at the water-silicone interface regions (not shown).

Figure 3.

Top, Emission spectra of 1,8-ANS in water-methanol mixtures and in acetonitrile. Also shown is the emission spectrum of 1,8-ANS in MyDay lens. The probe 1,8-ANS concentration in all the solvents used is same. Bottom, fluorescence intensity decays for the same samples. Excitation wavelength is 375 nm. IRF is the instrument response function.

Figure 4. Sodium-dependent emission spectra (top) and intensity decays (bottom) of SG-PL in MyDay contact lens. $\lambda_{\text{ex}} = 495 \text{ nm}$ and $\lambda_{\text{em}} = 545 \text{ nm}$. IRF is the instrument response function.

Figure 5. Binding affinity curves of SG-PL with sodium in Biofinity and MyDay lenses as measured by intensities at peak maximum (top) or amplitude-weighted lifetimes (bottom). Numerical values indicate the mid-points of the sodium response.

Figure 6. Reversibility of sodium binding to SG-PL in MyDay lens measured by intensities (top) or lifetimes (bottom).

Figure 7. Sodium-responses of SG-PL labeled MyDay lens in MOPS buffer and in the presence of 1mg/ml of HSA, Mucin or Lysozyme. Numerical values indicate the midpoints for respective sodium response curves. Top, intensity responses. Bottom, lifetime responses.

Figure 8. Emission spectra (top) and intensity decays (bottom) of the chloride sensitive probe OD-MQB in Biofinity lens with increasing chloride concentrations in pH 7.2 phosphate buffer. Excitation wavelength is 375 nm.

Figure 9. Emission spectra (top) and intensity decays (bottom) of OD-MQB in MyDay lens, with increasing chloride concentration, 20 mM MOPS, pH 7.2 buffer. Excitation wavelength is 375 nm.

Figure 10.

Stern-Volmer plots for chloride quenching of OD-MQB in Biofinity and MyDay contact lenses. Intensities (top) were measured at emission maxima, 454 nm (Figure 8 and 9). Lifetime values (bottom) were determined from fitting intensity decays to three exponential components and calculating the amplitude weighted average lifetimes, τ_α (see Table S5 and S6).

Figure 11. Absorption and emission spectra of OD-MQB in methanol and that of SG-PL in MOPS buffer, pH 7.2. Excitation wavelength for OD-MQB is 375 nm and it is 495 nm for SG-PL.

Figure 12.

Top, Emission spectra of double-labeled (OD-MQB and SG-PL) MyDay lenses with increased sodium chloride concentrations, 20 mM MOPS pH 7.2 buffer. Excitation wavelength is 360 nm. The emission band at 454 nm is from OD-MQB, and the emission at 550 nm is from SG-PL. Bottom, Intensity decays of double labeled lens with increasing concentrations of sodium chloride. Excitation wavelength is 375 nm and emission monitored at 445 nm (OD-MQB emission).

Figure 13.

Emission spectra (top) and sodium responses (bottom) of SG-PL in double-labeled (OD-MQB and SG-PL) MyDay lens. Excitation wavelength is 495 nm. SG-PL in MyDay lens intensity decays were measured at 545 nm with excitation of 490 nm. Measurements were performed in 20 mM MOPS (pH 7.2) buffer.

Figure 14.

(A) SG-PL response to sodium, (B) OD-MQB response to chloride, (C) SG-PL and OD-MQB double probe labeled lens response to NaCl with 375 nm excitation and (D) SG-PL and OD-MQB double probe labeled lens response to NaCl with 495 nm excitation. The insets show Boolean logic and truth tables for respective responses.

Table 1.

Contact Lens Properties

Polymer	Trade Name	Manufacture	Water (%)	Dk	Modulus (MP)
Comfilcon A	Biofinity	Cooper Vision	48	128	0.87
Stenfilcon A	MyDay	Cooper Vision	54	80	0.40