A Glucose sensing contact lens: A new approach to non-invasive continuous physiological glucose monitoring

Ramachandram Badugu[a], Joseph R. Lakowicz[a]* and Chris D. Geddes[a,b]*
[a]Center for Fluorescence Spectroscopy, Department of Biochemistry & Molecular Biology, University of Maryland School of Medicine, 725 West Lombard St., MD 21201, USA.
[b]Inistitute of Fluorescence and Center for Fluorescence Spectroscopy, Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 West Lombard St., MD, 21201, USA.

ABSTRACT

We have developed a new technology for the non-invasive continuous monitoring of tear glucose using a daily use, disposable contact lens, embedded with sugar-sensing boronic acid containing fluorophores. Our findings show that our approach may be suitable for the continuous monitoring of tear glucose levels in the range 50 – 500 μM, which track blood glucose levels that are typically ≈ 5-10 fold higher. We initially tested the sensing concept with well-established, previously published, boronic acid probes and the results could conclude the used probes, with higher pK_a values, are almost insensitive towards glucose within the contact lens, attributed to the low pH and polarity inside the lens. Subsequently, we have developed a range of probes based on the quinolinium backbone, having considerably lower pK_a values, which enables them to be suitable to sense the physiological glucose in the acidic pH contact lens. Herein we describe the results based on our findings towards the development of glucose sensing contact lens and therefore an approach to non-invasive continuous monitoring of tear glucose using a contact lens.

Keywords: Boronic acid probes, glucose, sensing, contact lens, non-invasive techniques.

1. INTRODUCTION

Diabetes results in long-term health disorders including cardiovascular disease and blindness. Close and continuous monitoring of glucose levels in the body is essential to reduce the adverse consequences of diabetes. A wide variety of methods have been proposed for the estimation glucose levels, including near infrared spectroscopy,[1,2] optical rotation,[3,4] colorimetric[5,6] and fluorescence detection.[7-11] The most commonly used technology for blood glucose determination is an enzyme-based method, which requires frequent blood sampling and therefore drawing. Although frequent "finger pricking" with a small needle to obtain the blood sample is a relatively painless process, this method does suffer from a few practical problems. The first one is inconvenience and the required compliance by patients, while the second is that this is not a continuous monitoring method. Despite intensive efforts, no method is presently available for the continuous non-invasive measurement of glucose.

Since the earliest reports of its presence in tears, glucose has remained a clinical and physiological curiosity[12,13]. Although there is both general interest and agreement that tear glucose levels are low, actual glucose concentrations remain confusing and somewhat contradictory[13,14]. For example, Giardini and Roberts have reported tear glucose concentrations to be approximately 3 mg/100 ml for subjects with normal glucose metabolism,[15] while Ridley[16] reported glucose concentrations of 65 mg/100 ml, where these discrepancies are now thought due to inappropriate sampling methods[13,14]. Elevated tear glucose during hyperglycemia was first demonstrated by Michail *et al.*[17,18] as early as 1937 as the tear glucose levels track blood levels, in an analogous manner to the equilibrium that normally exists for glucose between blood and tissue fluid.[19] Since that time tear glucose has been used on occasion to assess patients for hyperglycemia in an invasive and non-continuous manner.[13]

In this paper, we employ the notion of elevated tear glucose levels during hyperglycemia to investigate, the possibility of continuous monitoring of tear glucose and therefore blood glucose, using a disposable, *off-the-shelf*,

*cfs@cfs.umbi.umd.edu; phone 1-410-706-8409; fax 1-410-706-8408; http://cfs.umbi.umd.edu

contact lens.[20-22] By incorporating monosaccharide sensitive fluorescent probes within such a lens, we can indeed make progress towards this non-invasive approach for glucose monitoring.

As with any sensors, there are several issues that have to be addressed. The first is to identify suitable transduction elements, which in the presence of glucose, can report / produce suitable signals. The second is the design of the matrix to incorporate the transduction elements. For this, we have chosen an *off-the-shelf* disposable plastic contact lens, primarily because its physiological compatibility has already been assessed, and finally, the optimization of the sensor, with regard to sensitivity, response time, reversibility and shelf-life etc. The later two issues will be discussed throughout much of this paper. For the identification of suitable transduction elements, boronic acid has been known to have high affinity for diol-containing compounds such as carbohydrates,[23-25] where the strong complexation has been used for the construction of carbohydrate sensors,[26-33] transporters[34] and chromatographic materials.[35] Naturally, boronic acid compounds have been used for the synthesis of glucose sensors,[36-42] where we note the work of Shinkai,[36,37] Norrild,[38] Lakowicz [39-42] and Drueckhammer,[29] to name but just a few.

Boronic acids are weak Lewis Acids composed of an electron deficient boron atom and two hydroxyl groups, (**1** in Figure 1), which can interact with strong bases like OH^- to from the anionic boronate form (**2** in Figure 1), showing typically high pK_a around 9.[43,44] Boronic acids couple with diols to form a boronic acid diester group (**3** in Figure 1). The diol is linked covalently, and the reaction is fast and completely reversible.[44] In comparison to the boronic acid group, the boronic acid diester group shows higher acidity (p$K_a \approx 6$) due to a more electrophilic boron atom. The monophenylboronic acid group shows higher affinity for *D*-fructose with a smaller affinity for *D*-glucose with dissociation constants of ≈ 0.5 and 10 mM respectively.[44] The use of the boronic acid groups for sensing sugars is strongly dependent on the molecular geometry and the aromatic species where the boronic acid group is present, hence glucose sensitive probes can be made with a variety of affinities, in the mM range for blood glucose,[40-42] and in the μM range for tear glucose.

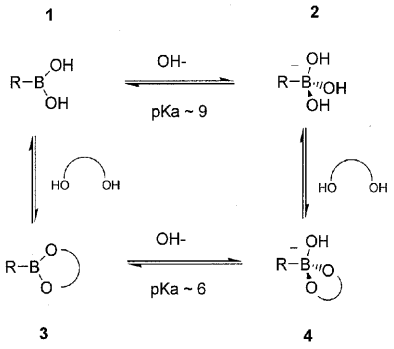


Fig. 1. Equilibrium for the Boronic Acid / Diol (Sugar) interaction

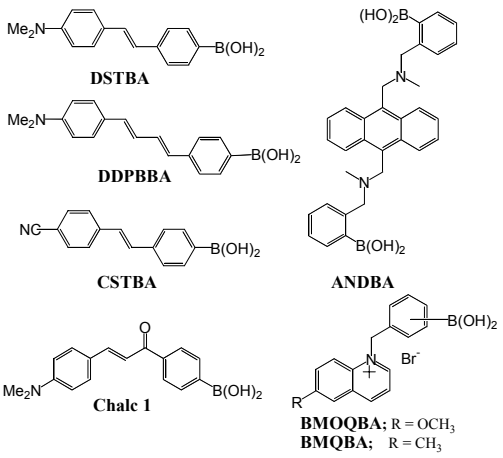
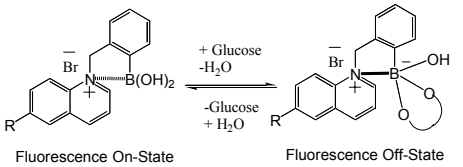


Chart 1- Molecular structure of the probes studied for use in contact lens

In this paper we report on Boronic Acid containing Fluorophores (BAFs, Chart 1) which employ different mechanisms to induce spectral changes in the presence of sugar in particular excited-state charge transfer (CT),[44] photoinduced electron transfer (PET)[37] and charge neutralization mechanism.[20-22] CT is a versatile mechanism that can be applied to a large number of fluorophores, where the boronic acid group and an electron donor group are present on the same fluorophore, in which the BA group [$-B(OH)_2$] acts as an electron withdrawing group. However, in the presence of sugar and at an appropriate pH, the boronic acid group is present in its anionic form, namely [$-B(OH)(Sugar)]^-$ and is no longer an electron withdrawing group. Hence spectral changes can be observed due to the perturbation of the charge transfer nature of the excited state. Here we employ this mechanism with a range of probes developed in our laboratory (Chart 1),[39-42,44-49] for glucose sensing within a plastic contact lens polymer. Photoinduced electron transfer mechanism is another extensively utilized mechanism in the monosaccharide sensing.[37] The change in the boronic acid conformation from neutral to anionic form with sugar binding alters the PET mechanism in the system and thereby the fluorescence spectrum. Compound ANDBA is a known PET probe selective for glucose with its geometrically preferred structure. The compatibility of the probe in contact lens is assessed. Before developing a new set of probes based on quinolinium nucleus (BMOQBA and BMQBA, Chart 1) we have made an attempt to understand the data obtained on CT and PET probes in contact lens along with the local pH and polarity of the contact lens. These new compounds with positively charged nitrogen center are highly fluorescent in aqueous solution. However, in the presence of monosaccharides the negatively charged boronate ester form resulted from the sugar complexation, partially neutralizes the charge density at



Scheme 1- A schematic representation of the sensing mechanism for the charge neutralization mechanism.

quaternary nitrogen center and thus leading to the fluorescence intensity drop (Scheme 1). A schematic representation of fluorescence sensing mechanism of these new sets of compounds is illustrated in scheme 1. These compounds, because of the charged nitrogen center, are highly water soluble and thus easy to use in physiological applications. Herein we report the spectral data obtained with the compounds presented in chart 1 in the contact lens, and compare with bulk solution based measurements to rational the design concept of a glucose sensing contact lens.

2. EXPERIMENTAL

2.1. Materials

All chemicals were purchased from Sigma. The preparation of the BAFs was in accordance with previous reports.[41,44, 50,51] The contact lenses were stirred several times in 500 ml water, 20°C for an hour before post-doping the probes. The contact lens is a polyvinyl alcohol type photocured polymer, which swells slightly in water. Its hydrophilic character readily allows for the diffusion of the aqueous analytes in tears. Doping was undertaken by incubating the lenses in a high concentration of the respective BAFs solution for 24 hrs before being rinsed in Millipore water. Lenses were used directly after being prepared.

2.2. Methods

All solution fluorescence measurements were undertaken in 4*1*1 cm fluorometric plastic cuvettes, using a Varian fluorometer. Doped contact lenses were mounted in a specially made lens holder (Fig. 2, left), which was itself inserted into a quartz cuvette having 1.5 mL pH 7.5 buffer for fluorescence sensing measurements. Excitation and emission was performed using a Varian Fluorometer, with the concave edge of the lens facing towards the excitation source (Fig. 2, right). This geometry was employed to reduce any scattering of the excitation light. We additionally tested the lens excited from the convex edge, just as would be used in the eye, and found identical results.

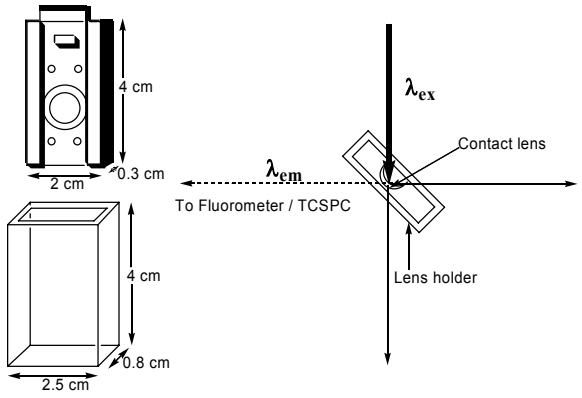


Fig. 2. Contact lens mount and quartz holder (left). The experimental geometry used in the sensing study using lens (right).

The quartz lens holder has dimensions of 4*2.5*0.8 cm, all 4 sides being of optical quality. The contact lens is mounted onto a stainless steel mount of dimensions 4*2*0.3 cm, which fits tightly within the quartz outer holder. A circular hole in the center of the mount with a 1.5 cm ID, has a raised quartz lip, which enables the lens to be mounted. The mount and holder readily allow for ≈ 1.5 cm^3 of solution to be in contact with the either sides of the lens for the sugar sensing experiments, Figure 2.

Leaching of the probes from the contact lens polymer was observed using the sample holder shown in Figure 2, which contained ≈ 1.5 cm^3 buffer, 20^0 C. A Varian fluorometer measured the intensity change as a function of time to determine the percentage signal change, corresponding to dye leaching. It should be noted that with no sample present, no intensity fluctuations or drifts were observed, indicating stability of the fluorometer Xenon-arc source.

2.3. Data Analysis

Titration curves against pH were determined in buffer solution: pH 3 and 4 acetate buffer; pH 5 to 9 phosphate buffer and pH 10 and 11 carbonate buffer. Titration curves were fitted and pK_a (pK_a = -Log_{10} K_a) values were obtained using the relation:

$$I = \frac{10^{-pH} I_{acid} + K_a I_{base}}{K_a + 10^{-pH}} \qquad (1)$$

where I_{acid} and I_{base} are the intensity limits in the acid and base regions respectively.

Stability (K_S) and dissociation (K_D) constants were obtained by fitting the titration curves with sugar using the relation:

$$I = \frac{I_{min} + I_{max} Ks[sugar]}{1 + Ks[sugar]} \qquad (2)$$

where I_{min} and I_{max} are the initial (no sugar) and final (plateau) fluorescence intensities of the titration curves, where $K_D = (1/K_S)$.

3. RESULTS

To determine the usefulness of BAFs in the estimation of the tear glucose using a contact lens, it is necessary to compare both solution and lens based measurements. All the solution based measurements were performed in pH 8.0 buffer-methanol (2:1) for CT and PET probes and pH 7.5 buffer was used for the quinolinium type probes. Doped contact lenses, which were previously washed and allowed to leach excess dye for 1 hr, were inserted in the contact lens holder, Figure 2. Buffered solutions of sugars were then added to the lens, which were also similarly buffered in the 1.5 cm^3 cell volume. Fluorescence spectra were typically taken 15 mins after each sugar addition to allow the lens to reach equilibrium.

3.1. Response of CT and PET probes

3.1.1. Charge transfer probes

Chart 1 shows 4 CT probes: two stilbene derivatives, one polyene derivative and one chalcone derivative. Compound 4'-Dimethylaminostilbene-4-boronic acid (DSTBA) combines the electron-donating dimethylamino group with the electron withdrawing boronic acid group, and 4'-Cyanostilbene-4-boronic acid (CSTBA) combines the electron withdrawing cyano group with the boronic acid, in essence two probes demonstrating both reduced and increased CT, respectively, in the presence of sugar. Also to test the suitability of longer wavelength probes in the contact lens we have considered a polyene derivative, DDPBBA, 1-(*p*-boronophenyl)-4-(*p*-dimethylaminophenyl)buta-1,3-deine,[40,44,45] which is structurally similar to that of DSTBA (Chart 1). Chalcone derivatives, unlike the stilbenes and polyenes, have the advantage of much longer wavelength emission. This is particularly attractive as longer wavelength emission reduces the detection of any lens or eye autofluorescence as well as scatter (λ^{-4} dependence), and also allows the use of cheaper and longer wavelength laser or light emitting diode excitation sources, reducing the need for UV excitation in the eye. Subsequently, we included a Chalcone derivative in our contact lens feasibility studies, which was previously synthesized in our laboratory namely, Chalc 1 (Chart 1).[42,44] For this probe, the boronic acid group does not produce resonance forms with the electron donating amino group. The CT occurs between the dimethylamino group (electron donating group) and the carbonyl group (electron withdrawing group). Upon sugar binding to the boronic acid group, then a change in the electronic properties of the boron group, both when free and when complexed with sugar, leads to a change in the electronic density of the benzophenone moiety and subsequently the CT properties of the excited state of the fluorophore, noting that boronic acid group is in resonance with the carbonyl group.

Emission spectra of DSTBA in pH 8.0 phosphate buffer-methanol (2:1, v/v) and in contact lens are shown in figure 3A and 3B, respectively. The emission spectrum in solution shows a hypsochromic shift of about 30 nm and an increase in fluorescence intensity with increase in glucose concentration. These dramatic and useful changes in the emission spectra of DSTBA in solution can simply be explained by the loss of the electron withdrawing property of the boronic acid group following the formation of the anionic form as shown in Figure 1. However, the response of DSTBA from doped contact lens is opposite, emission intensity of the probe steadily decreases with increasing glucose concentration associated with negligible/no shift in the band maximum (Fig. 3B). The corresponding ratiometric plot for DSTBA in buffer and lens was plotted and shown in figure 3C. The binding affinity towards glucose in lens is slightly decreased. The lack of suitable spectral shifts in the presence of sugar eliminates, at this stage, the possibility of wavelength ratiometric sensing as shown for the solution based measurements in Figures 3A.

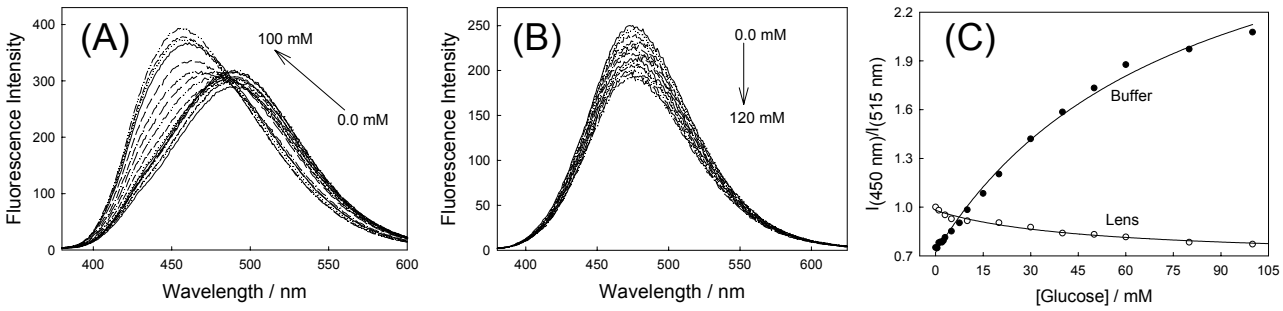


Fig. 3- Emission spectra of DSTBA in pH 8.0 phosphate buffer-methanol (2:1) with glucose (A), in lens (B), λ_{ex} = 340 nm. Ratiometric response of the probe in buffer and lens with glucose (C).

The other stilbene derivative, CSTBA, possesses two electron-withdrawing groups. In the presence of sugar one can observe a bathochromic shift, about 25 nm, and a decrease in fluorescence intensity at pH 8, (data not shown) which is

opposite of that observed for DSTBA.[20] This change has been attributed to an excited CT state present for the anionic form of CSTBA, where no CT states are observed for the neutral form of the boronic acid group,[44] suggesting that the anionic form of the boronic acid group can act as an electron donor group. Similarly, CSTBA in lens shows reduction in fluorescence intensity with increasing glucose as compared to solution; no red shift in the emission is observed, indicative of a reduction in the electron donating capability of the anionic sugar bound form in contact lens.[20] It is interesting to see the much greater response for fructose for CSTBA in the lens as compared DSTBA, where notable changes in intensity occur at < 20 mM [fructose]. However the glucose response of DSTBA in the contact lens appears more promising for [glucose] < 10 mM, where a 10 % fluorescence intensity change is observed for ≈ 10 mM glucose at pH 8.0.

In order to test the suitability of longer wavelength probes in the contact lens we also considered a polyene derivative, DDPBBA, 1-(*p*-boronophenyl)-4-(*p*-dimethylaminophenyl)buta-1,3-deine,[40,44,45] which is structurally similar to that of DSTBA (Chart 1). As was observed for DSTBA, DDPBBA shows blue-shift in the emission and an increase in intensity with increasing glucose concentrations (Fig. 4A). On the other hand as shown in figure 4B the response of DDPBBA towards glucose in contact lens is very small, except a small decrease in intensity, which is very similar to that of with DSTBA in lens.

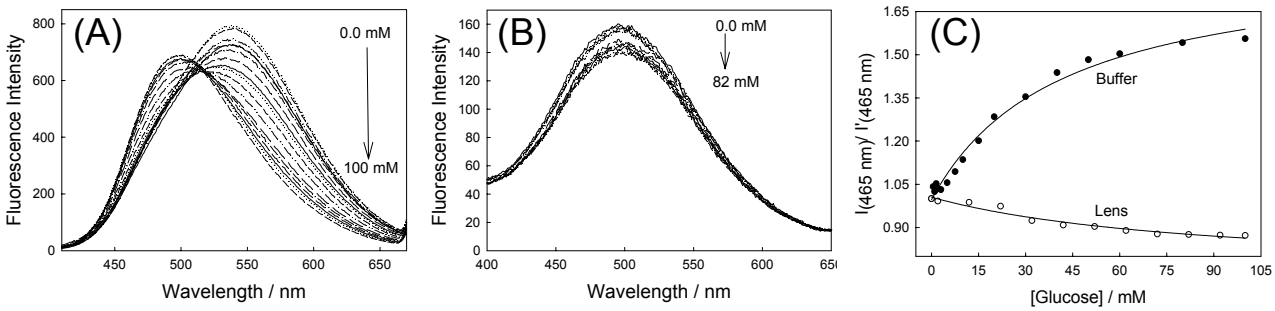


Fig. 4- Emission spectra of DDPBBA in pH 8.0 phosphate buffer-methanol (2:1) with glucose (A), in lens (B), λ_{ex}= 340 nm. Ratiometric response of the probe in buffer and lens with glucose (C).

Emission spectra of Chalc 1 shows an emission centered around 580 nm and about 5 folds increase in intensity with 50 mM fructose. But in the lens it shows an emission band centered at 560 nm (data not shown). In contrast to the responses observed in solution, a reduction in fluorescence intensity is observed for Chalc 1 doped contact lens.[20] Almost all the CT probes, although they show excellent response towards glucose in solution, are insensitive in the contact lens.

3.1.2. PET probe

Compound ANDBA (Chart 1) is a well-established PET probe, which selectively binds glucose.[37] Emission spectra of ANDBA in pH 8.0 phosphate buffer-methanol (2:1) and in lens with glucose are presented in Figure 5A and 5B, respectively. The response of the probe with glucose in buffer and lens with glucose is shown in figure 5C. As it is discussed in the literature,[37] the increase in emission intensity of ANDBA in solution with glucose is attributed to the suppression of the PET quenching, originating from amino nitrogens to anthracene, due to the interaction between the boron and nitrogen atoms following the binding with glucose. On the other hand, ANDBA response towards glucose in lens is insignificant (Figs. 5B and 5C).

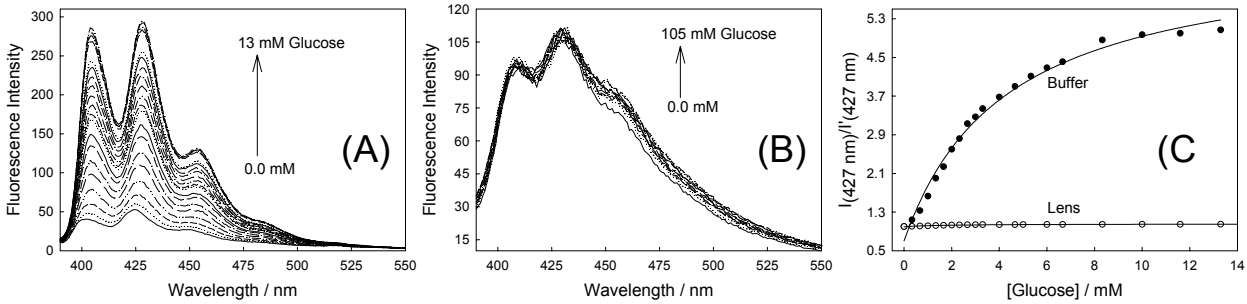


Fig. 5- Emission spectra of ANDBA in pH 8.0 buffer-methanol (2:1) with increasing concentrations of glucose (A), in lens (B), λ_{ex} = 340 nm, and response with glucose in buffer and lens (C).

3.2. Charge neutralization based probes

The difference in the signal transduction of the CT and PET probes, discussed above, in buffer and lens can be explained considering several factors that influence the initial and final CT or PET efficiency in the system, the binding affinities of the boronic acid moieties with glucose in two different media and also the rate of glucose diffusion into the relatively less polar lens. The estimated polarity of the lens, which is very similar to that of methanol, and a pH of about 6.0, are important aspects to consider in developing a boronic acid probe for tear glucose monitoring. Especially, as all the probes discussed so far have relatively high pK_a vales, of about 9 in the absence of sugar, as the low pH inside the contact lens may destabilize the boronate diester complex. Subsequently, we considered a logical strategy to develop boronic acid probe with reduced pK_a values. Generally, pK_a value of phenylboronic acid is tunable by the suitable substitution effects. For example, an electron acceptor group on the ring lowers the pK_a of boronic acid where as a donor group increases. In this regard, we have considered the interaction of a quaternary nitrogen atom (an efficient acceptor) with boronic acid in the design of a new set of probes for glucose sensing that compatible in contact lens. Subsequently, we have prepared a set of compounds BMOQBAs and BMQBAs (BMOQBAs: *o*-BMOQBA–N-(2-boronobenzyl)-6-methoxyquinolinium bromide, *m*-BMOQBA–N-(3-boronobenzyl)-6-methoxyquinolinium bromide, *p*-BMOQBA–N-(4-boronobenzyl)-6-methoxyquinolinium bromide, and BMQBAs: *o*-BMQBA–N-(2-boronobenzyl)-6-methylquinolinium bromide, *m*-BMQBA–N-(3-boronobenzyl)-6-methylquinolinium bromide, *p*-BMQBA–N-(4-boronobenzyl)-6-methylquinolinium bromide) based on the quinolinium nucleus. All six probes were prepared in a one step synthesis reported previously by us.[50,51] All the probes are readily water-soluble and have high quantum yields.

3.2.1. Response towards pH

Fluorescence emission spectra of *o*-BMOQBA in various pH solutions are shown in figure 6. As it can be seen from the figure, the fluorescence intensity of the probes drops steadily with increasing pH. The corresponding pH response plots obtained by normalized emission intensities at the band maximum for BMOQBAs and BMQBAs are shown in figures 6B and 6C, respectively. The pK_a values obtained using equation 1 for compounds BMOQBAs and BMQBAs along with CT and PET probes are depicted in Table 1. As was expected the pK_a values of the new probes BMOQBAs and BMQBAs are considerably less than those of the other probes discussed in this paper. For example glucose bound boronic acid pK_a for BMOQBAs and BMQBAs are less than 7, which is in the range of contact lens pH.

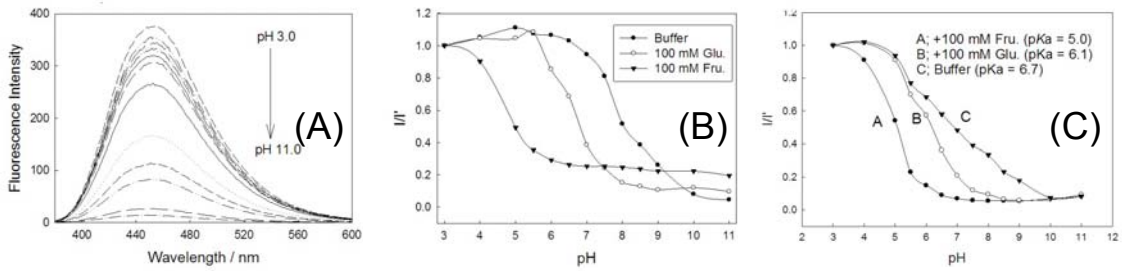


Fig. 6- Emission spectra of *o*-BMOQBA in buffer media (A), λ_{ex} = 345 nm. Emission intensity at band maximum, *I,* divided by the initial intensity, *I'*, for *o*-BMOQBA (B), and *o*-BMQBA (C).

Table 1. pK_a values for different probes studied in contact lens and effect of saturated amount of sugars.

Probe	In Buffer	+ 100 mM Glucose	+100 mM Fructose
***o*-BMOQBA**	7.90	6.62	4.80
***m*-BMOQBA**	7.70	6.90	5.00
***p*-BMOQBA**	7.90	6.90	5.45
***o*-BMQBA**	6.70	6.10	5.00
***m*-BMQBA**	7.75	6.85	5.05
***p*-BMQBA**	7.80	6.95	5.45
DSTBA	9.14[a]	8.34	6.61
DDPBBA	8.90[a]	6.97	6.20
CSTBA	8.17[a]	7.30	5.84
Chalc 1	7.50[b]	---	5.40
ANDBA	11[c]	---	---

[a]from ref. 45, [b]ref. 42, and [c]ref. 37.

3.2.2. Response towards sugar in buffer and contact lens

Figure 7A shows the fluorescence emission spectra of *o*-BMOQBA in pH 7.5 buffer with increasing concentrations of glucose. As the glucose concentration increases we typically see a reduction in the fluorescence intensity at 457 nm. The normalized emission intensity at band maximum plotted versus glucose concentration is shown in the figure 7B and from which we can see an about 2.8 fold change in intensity by the addition of 60 mM glucose. Interestingly, figure 7B shows useful intensity changes in the presence of physiologically important glucose concentrations, where the blood glucose level is 3-8 mM for a healthy person and increases to between 2 and 40 mM in diabetics.[44] In the tear glucose range (μM glucose concentration range), Figure 7C, we are able to see a ≈ 10-15 % change in fluorescence intensity by the addition of 2 mM glucose, where as fructose shows a much greater response in this concentration range (data not shown). As well as considering the differences in sugar affinities for a given probe in solution, we can also compare the differences in sugar affinities between the 3 different isomers, Table 2. The *o*-BMOQBA shows a greater response to glucose and the *p*-BMOQBA towards fructose, with a notable change in fluorescence intensity for glucose concentrations less than 10 mM. Similarly, the addition of 10 mM fructose shows a ≈ 6 - fold change in intensity.[20] The data in Figure 7 can be used with equation 2 to determine the dissociation constants, Table 2, which roughly reflects these visual trends. However, while equation 2 is routinely used to obtain boronic acid – sugar dissociation and binding constants[36-42], we found only a modest confidence in the fitting procedure here, illustrated by comparing the visual trends in Figure 7 with the recovered values in Table 2. While beyond the scope of this text, these fitting difficulties clearly reflect the need for a new kinetic sugar binding function with our new probes. Further studies are underway in this regard.

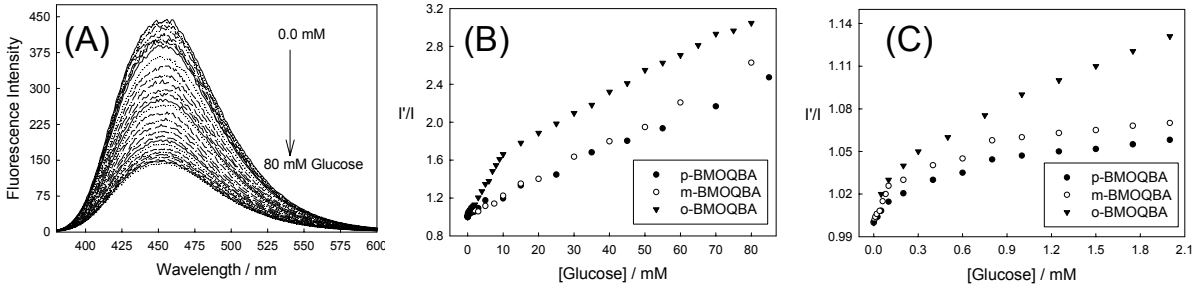


Fig.7- Emission spectra of *o*-BMOQBA in pH 7.5 phosphate buffer with glucose (A), λ_{ex} = 345 nm. The respective 450 nm intensity ratio in the absence, *I'*, and in the presence of glucose, *I*, for all three isomers (B), and in the low concentration range of the glucose (C).

Figure 8A shows the emission spectra of *o*-BMOQBA doped contact lens towards glucose. The doped lens clearly shows good responses towards both sugars. We again constructed the *I'/I* plots, where *I'* is the intensity in the absence of sugar and *I* in the presence, Figures 8B and 8C. The response towards fructose was greater at high sugar concentrations, however, in the low concentration range glucose and fructose have a similar affinity in the lens, with a ≈ 20 % change in fluorescence signal with the addition of only 500 μM glucose.[21] Clearly we see a comparable response in lens towards sugars with that of solution based studies at pH 7.5 (see figures 7B and 7C and Figures 8B and 8C). This wasn't unexpected, and is simply explained by the pK_a of the probes being < 7. More difficult to explain however is the similar affinity of both glucose and fructose in the lens at low sugar concentrations, which we can only attribute to the presence of the lens at this time. We repeated these doped lens experiments several times, and in all cases the trends were reproducible.

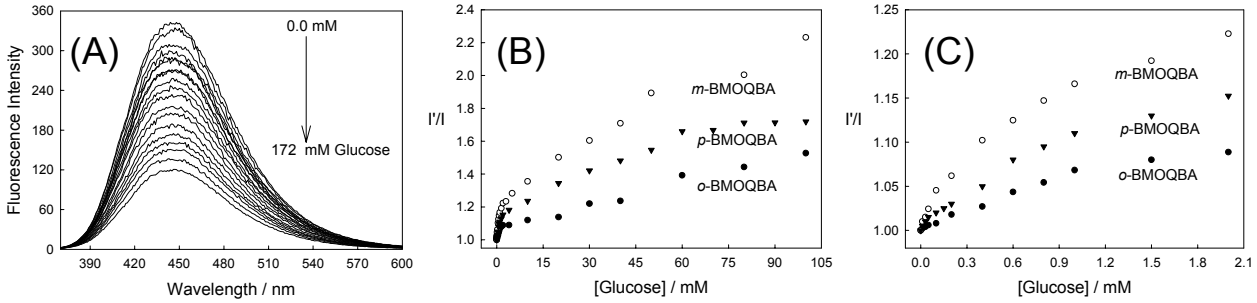


Fig. 8- Emission spectra of *o*-BMOQBA in contact lens with glucose (A), λ_{ex} = 345 nm. The respective 450 nm intensity ratio in the absence, *I'*, and in the presence of glucose, *I*, for all three isomers (B), and in the low concentration range of the glucose (C).

Very similar to that of BMOQBAs, BMQBAs show a comparable response towards glucose in pH 7.5 buffer and in a contact lens.[22] Figures 9A and 10A show the corresponding emission spectra in buffer and lens. As was the case with BMOQBAs as the glucose concentration increases we typically see a reduction in the fluorescence intensity at 427 nm. The normalized emission intensity at band maximum plotted versus glucose concentration is shown in the figure 9B and 10B and from which we can see an about 2.0-2.4 fold change in intensity by the addition of 60 mM glucose. In the tear glucose range, Figure 10C, we are able to see a ≈ 10-20 % change in fluorescence intensity by the addition of 2 mM glucose. All three isomers of BMQBA show a very similar response either in buffer or in lens. The dissociation constants, similarly obtained using equation 2, are presented in Table 2 for comparison. The binding affinities are relatively better for the BMQBAs compared with the BMOQBAs.

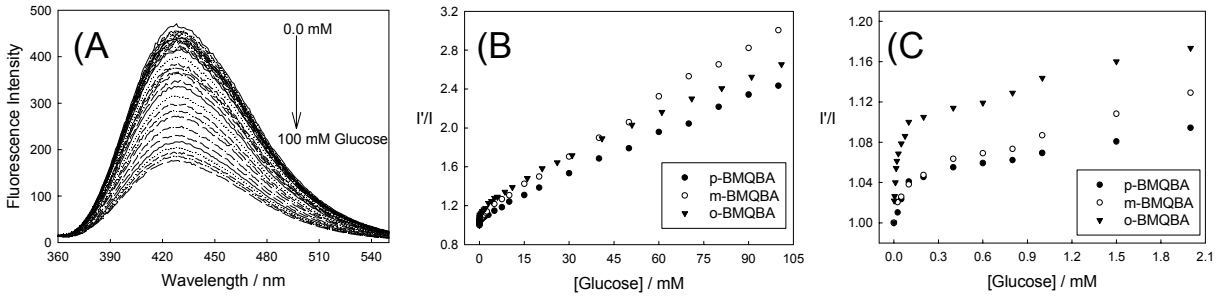


Fig. 9- Emission spectra of *o*-BMQBA in pH 7.5 phosphate buffer with glucose (A), λ_{ex} = 320 nm. The respective 427 nm intensity ratio in the absence, *I'*, and in the presence of glucose, *I*, for all three isomers (B), and in the low concentration range of the glucose (C).

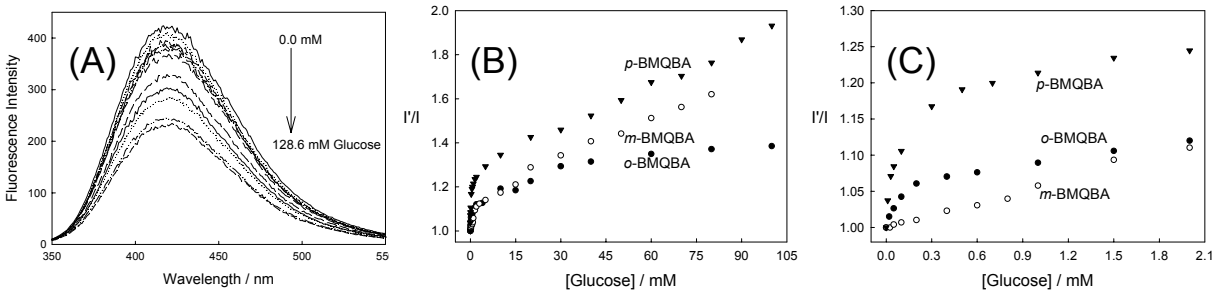


Fig.10- Emission spectra of *o*-BMQBA in contact lens with glucose (A), λ_{ex} = 320 nm. The respective 427 nm intensity ratio in the absence, *I'*, and in the presence of glucose, *I*, for all three isomers (B), and in the low concentration range of the glucose (C).

Table 2. Dissociation constants, K_d (mM), for BMOQBAs and BMQBAs with glucose and fructose in buffer and lens.

Probe	**Glucose**		**Fructose**	
	Buffer	***Lens***	***Buffer***	***Lens***
***o*-BMOQBA**	49.5	322.6	0.65	84.7
***m*-BMOQBA**	1000	54.6	1.8	4.9
***p*-BMOQBA**	430.0	111.1	9.1	34.7
***o*-BMQBA**	100	17.9	4.7	34.8
***m*-BMQBA**	476	58.1	13.2	21.6
***p*-BMQBA**	370	128.2	13.8	12.9

4. DISCUSSION

It is generally found that when designing and fabricating plastic sensors, both the transduction element and polymeric support are either chosen simultaneously based on the properties of both, or a support is found that is compatible with the sensing probes and the environment to be sensed. Seldom do we see a sensor whose transduction elements are chosen based on the merits of the polymeric support alone. However, because the physiological compatibility of disposable plastic contact lenses has already been assessed and optimized, with regard to vision correction, size and oxygen / analyte permeability etc, we are encouraged to design and fabricate a glucose sensing

contact lens starting with the unmodified polymeric support. Subsequently, we chose a range of water-soluble BAFs that have been previously synthesized and well-characterized by our laboratory and others,[39-42,45-49] to assess the feasibility of a glucose sensing contact lens based on BAFs.

The initial reports and binding constants of these BAFs suggested the possibility of detecting sub mM glucose concentrations[39-42,45-49] which is required for tear glucose monitoring.[12-14,19,52] However, we have found the response of the BAFs was notably different in the lens as compared to solution, although given their spectral differences alone, then this doesn't preclude their use in a glucose sensing contact lens.

For CT and PET probes studied, the response to sugars in the lens was opposite to that observed in solution. To understand these changes and therefore characterize the contact lens environment for further sensor development, we assessed the response of the BAFs in solution in both different pH and polarity media. The emission spectra of DDPBBA in different pH media in the presence of increasing glucose concentrations were studied.[20] Interestingly, we were able to observe a similar spectral response to glucose in pH 6.0 media as compared to the contact lens, although the wavelength emission maxima are somewhat different suggesting more than just a pH effect. At this pH it is thought that the sugar bound form would be dominant, p$K_a \approx 6$. Indeed, in a study of all the probes in both solution and in the contact lens, we observed lens responses that were not identical to those observed in any pH solution, again suggesting an additional effect is also playing a role on the BAFs spectral response to sugar in the contact lens. Recently, we have investigated the lens pH further by doping a contact lens with the well-known pH sensitive probe, fluorescein, and measured the fluorescein lifetime/s in the lens, determined using the time-correlated single photon counting technique.[20] A comparison with the lifetimes obtained for fluorescein in different pH buffers led us to conclude a lens pH in the range 5.5 – 6.5. Also, we assessed the polarity within the contact lens, by measuring the intensity ratio of the 0,0 (or I_1) and 0,2 (or I_3) bands of a pyrene doped lens,[20] where the intensity ratio of pyrene fluorescence bands is widely used to estimate the polarity of media, such as in micelles.[54,55] The lens was post-doped with pyrene by immersing the lens in a pyrene buffer methanol solution (pH 8.0, 2:1 v/v) for 1 hr, and then rinsed extensively with Millipore water. The estimated value of I_1 / I_3 was ≈ 1.28, indicating the polarity within the lens is not indifferent than that of methanol (I_1 / I_3 for MeOH = 1.33).[55] In retrospect this was not surprising given that the contact lens is PVA based.

By determining both the pH and polarity within the contact lens it is possible to rationale the different spectral responses we have observed as compared to solution. As the solution pH increases the emission spectrum of DDPBBA displays a large blue shift.[20] These spectral changes induced by the pH are due to the formation of the anionic form of the boronic acid group (form 2 in Figure 1). As the anionic boronate species is formed, the boron group is no longer an electron-withdrawing group, resulting in the removal and/or perturbation of the charge transfer nature of the excited state. An important feature here is the change in acidity (electrophilicity) of the boron group between the uncomplexed and complexed forms. Indeed this acidity change is the driving force enabling the use of the boronic acid moiety for sugar sensing. At a lower pH (such as in the contact lens), the simple complexation of the boronic acid with sugar (the equilibrium between species 1 and 3 in Figure 1) does not fully result in a perturbation of the fluorophore, hence DDPBBA is not suitable as a wavelength ratiometric probe in the contact lens. The same however would be true at a much higher pH also, i.e. the equilibrium between species 2 and 4, Figure 1. To induce a spectral change of the fluorophore, the complexation of the BAFs with sugar should result in a perturbation of the electronic properties of the fluorophore, i.e. from the neutral (1 in Figure 1) to the anionic form (4 in Figure 4). As was briefly mentioned in the introduction, these BAFs typically display pK_a around 9, with a p$K_a \approx 6$ for the sugar complexed form, Figure 1.[44] Hence these probes are ideal for solution sugar sensing in the pH range 6.5-8.5, which for blood glucose levels is ideal,[52] where the maximum spectral change is usually observed in the pH range 7 – 7.5. However, the low pH nature of the contact lens limits the spectral changes and therefore the dynamic range for tear glucose sensing. In addition these probes are polarity sensitive. For DSTBA and DDPBBA, as the polarity of the solvent increases, a red-shifted emission band can be observed (see Figure 3 in ref 44), which accounts for the emission maximum difference between DDPBBA in the contact lens. Similar rationale can also be drawn for the other probes considered here.

Based on these findings it appears that to observe suitable spectral responses in the presence of sugars within the contact lens, and therefore to maximize the dynamic range for tear glucose sensing, then either a method of controlling the pH within the lens must be adopted, noting that our attempts of external buffering were unsuccessful, or else probes with lower pK_a need to be used. Given that BAFs have mostly been designed for blood glucose measurements at ≈ pH 7.5,[44] it is likely that we will need to both design and synthesize suitable BAFs.

With this aim, we have designed the probe molecules based on the quinolinium moiety as the reporter, and the phenylboronic acid as the binding site for monosaccharide. The quinolinium nitrogen in these systems not only lowers the pK_a values (Table 1) but also stabilizes the boronate diester formed with sugar, affording them as suitable probe molecules to be used in the predetermined polymeric contact lens support. Unlike CT and PET probes, BMOQBA and

BMQBA probes show comparable response in solution and in the lens, and about 15-20 % change in intensity is observed within the tear glucose concentration range.[21,22]

5. CONCLUSIONS

We have tested the feasibility of physiological glucose sensing in tears, which is known to track blood glucose levels approximately 10 fold, using a disposable and *off-the-shelf* plastic contact lens embedded with boronic acid containing fluorophores. While the solution response of the boronic acid containing fluorophores shows the feasibility of determining the required low glucose concentrations, the low contact lens pH and methanol-like polarity significantly reduces the dynamic range for sensing. Subsequently we can conclude that this approach for the potential continuous monitoring of glucose is feasible if suitable boronic acid containing fluorophores can be designed to respond well in the contact lens micro environment.

Subsequently, we have synthesized and tested a range of new boronic acid containing fluorophores that are compatible with the low pH and methanol-like polarity within a contact lens. We have developed a proto-type glucose sensing contact lens based on embedded boronic acid containing fluorophores that responds well to glucose concentrations in the tear range, 50 – 500 μM glucose. We have shown sensor responses of ≈ 20 % for glucose in this range, which would readily enable normal glucose levels of a healthy person to be determined, where elevated tear glucose levels during hyperglycemia would inevitably produce an even greater signal response. In addition our prototype has a 90 % response time of about 10 minutes, does not leach probe and has a shelf-life in excess of the several month experimental period.

We also believe that this approach may well offer an alternative sensing platform to current invasive glucose monitoring techniques, where simple spectroscopy can be used to determine the response of suitable fluorescent probes towards glucose, in the contact lens within the eye. In addition the notion of using a contact lens to sense glucose is likely to be well received by diabetics, as many have eye disorders and require vision correction, which is thought to be due to glycosylation of protein in blood vessels.[53]

With diabetes being widely recognized as one of the leading causes of death and disability in the western world, we believe our boronic acid doped contact lens approach and findings, are a notable step forward towards the continuous and non-invasive monitoring of blood glucose. As well as tear glucose, other tear analytes could also be determined by the incorporation of suitable transduction elements within contact lenses, such as chloride or even cholesterol. Further reports of physiological analyte monitoring using this non-invasive contact lens approach will be reported by our laboratory in due course.

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