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# Photonic Crystal Glucose-Sensing Material for Noninvasive Monitoring of Glucose in Tear Fluid

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***Background:*** **We recently developed a photonic crystal glucose-sensing material (Alexeev et al., *Anal Chem* 2003;75:2316–23), which consists of a crystalline colloidal array embedded within a polymer network of a polyacrylamide-poly(ethylene glycol) hydrogel with pendent phenylboronic acid groups. The aim of the present work was to improve this approach for application to noninvasive or minimally invasive monitoring of glucose.**

***Methods:*** **We used new boronic acid derivatives such as 4-amino-3-fluorophenylboronic acid and 4-carboxy-3-fluorophenylboronic acid as the molecular recognition elements to achieve sensing at physiologic pH values.**

***Results:*** **The improved photonic glucose-sensing material sensed glucose in the range of the 100 μmol/L concentrations found in tear fluid. The detection limits were ~1 μmol/L in synthetic tear fluid. The visually evident diffraction color shifted across the entire visible spectral region from red to blue over the physiologically relevant tear-fluid glucose concentrations. This sensing material is selective for glucose over galactose, mannose, and fructose.**

***Conclusions:*** **These new glucose sensors have properties appropriate for use in such glucose-sensing applications as ocular inserts or diagnostic contact lenses for patients with diabetes mellitus.**



The incidence of diabetes mellitus is increasing to epidemic proportions in the United States and the developed world *(1)*. The Diabetes Control and Complications Trial *(2)* clearly demonstrated that glycemic control in diabetes management in patients is crucial. This is recognized in the current standards of care set forth by the American Diabetes Association. Control of blood glucose can avoid the negative health consequences of diabetes mellitus *(3)*; there thus is an ever increasing need for continuous, noninvasive glucose monitoring for people with diabetes mellitus.

A critical component of intensive diabetes management is accurate and frequent home glucose monitoring, but the current generation of home glucose meters are accurate only to ±15%, require a fingerstick for blood sampling, and must be carried everywhere with the patient. The pain of the fingerstick severely reduces patient compliance with frequent glucose monitoring.

Real-time noninvasive, painless sensing devices that could continuously monitor the glucose concentration in blood or in a surrogate bodily fluid whose glucose concentration is tightly linked to the glucose concentration in blood would have considerable impact. The ideal device should allow simple determination with immediate feedback regarding blood glucose concentration. This would provide for greatly improved glycemic control.

The pressing demand for noninvasive glucose sensors has stimulated the development of a wide variety of sensing approaches, which were reviewed recently *(4–6)*. In the most recent approach, we demonstrated a potentially revolutionary method for continuous glucose monitoring that uses a photonic crystal that responds to physiologically relevant glucose concentrations *(7, 8)*. Our sensor diffracts light in a narrow wavelength band (color) in the visible spectrum; the diffracted band shifts as the glucose concentration changes. This diffracted wavelength can be observed visually and can be used to determine the glucose concentration of the surrounding fluid.

We envision that this new sensor material could be used for noninvasive glucose sensing in tear fluid. The sensor would be contained in contact lenses or in ocular inserts designed for use under the lower eyelid (Fig. 1). Patients would determine their glucose concentration by viewing the color of the sensing material by use of a compact device that contains a white light source, a

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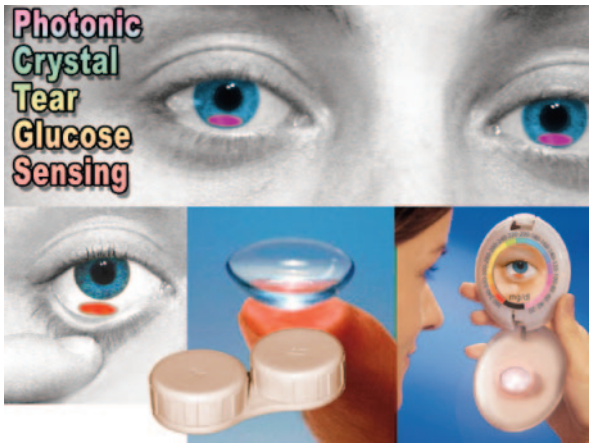



Fig. 1. Pictorial representation of use of glucose-sensing material to determine glucose concentration in tear fluid.

The photonic crystal sensing materials would be contained in a contact lens or ocular insert. The color diffracted changes with the tear glucose concentration. A simple mirrored compact-like device would illuminate the sensor material with white light. The color of the sensor would be determined by viewing the reflected (diffracted) light and comparing it with an exterior color wheel calibrated in terms of the blood glucose concentration.

mirror, and a color chart. The observed color would indicate the glucose concentration in the patient's tear fluid. Alternatively, a simple spectral measuring instrument could be used to measure the diffracted wavelength; this instrument would calculate and display the blood glucose concentration. Because tear-fluid glucose concentrations parallel blood glucose concentrations *(9–12)*, a tear-fluid glucose measurement could be used to determine the blood glucose concentration.

The sensing material consists of a face-centered cubic array of colloidal particles embedded in a hydrogel (Fig. 2). The array spacing is ~250 nm, designed such that the array diffracts visible light. We previously demonstrated

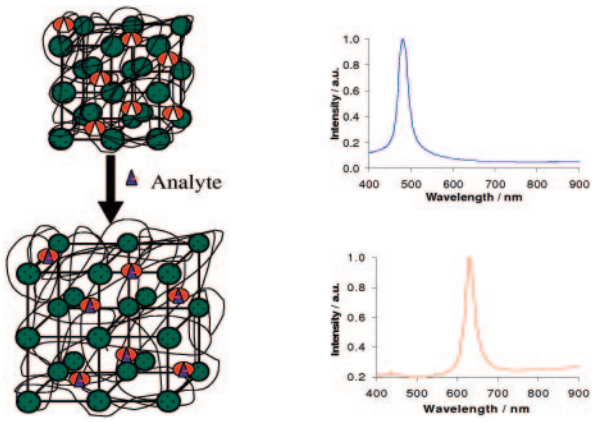



Fig. 2. PCCA photonic crystal sensing materials consist of an embedded CCA surrounded by a polymer hydrogel network that contains a molecular recognition element.

The embedded CCA of polystyrene particles diffracts light of a wavelength determined by the array lattice constant. As shown by the spectra on the *right*, a diffracted wavelength redshift results from hydrogel volume swelling induced by interaction of the analyte with the molecular recognition element. *a.u.*, absorbance unit(s).

that by attaching molecular recognition agents, we could use this versatile sensor platform to sense numerous analytes, such as glucose, creatinine, cations, pH, and ionic strength *(13–15)*.

For glucose sensing, we attach boronic acid recognition agents to bind to glucose to produce cross-links that blueshift the diffraction in proportion to the glucose concentration. Recently, we used this sensing motif to sense glucose in low-ionic-strength pH 8.5 aqueous solutions *(7)* and in pH 8.5 and 7.4 high-ionic-strength aqueous solutions *(8)*.

Our use of boronic acid groups was inspired by numerous previous reports on the binding to boronic acid of diol-containing species such as carbohydrates *(16–20)*. For example, Kikuchi et al. *(16)* previously demonstrated actuation of hydrogel volume changes by boronic acid-sugar complexation. When glucose binds to them, phenylboronic acid-poly(vinyl alcohol) hydrogels swelled, which led to increased diffusion of ionic species and increased ion currents. More recently, Arnold et al. *(21)* described a glucose conductimetric detection scheme that uses the release of protons resulting from glucose-boronic acid complexation. In addition, carbohydrate-induced boronic acid hydrogel swelling was used in a quartz crystal microbalance carbohydrate-sensing scheme *(17–19)*. Interested readers should consult the recent reviews by James and coworkers *(22, 23)*, Wang et al. *(24)*, and Striegler *(25)* to more fully review recent developments in boronic acid-based carbohydrate sensors.

Recently we described a photonic crystal glucose-sensing material that operated at physiologic pH (7.4) and ionic strengths *(8)*. This sensor was fabricated from a novel fluorinated boronic acid derivative *(26)* with a p$K_a$ lower than that typical of aromatic ring-substituted boronic acid derivatives. This lower p$K_a$ boronic acid derivative is essential for a high-sensitivity glucose-sensing material because high-affinity glucose binding requires a significant concentration of boronate species with high glucose affinity; the acid-base chemistry of glucose binding is quite complex, as shown in Scheme 1 *(27–29)*. In fact, it would be desirable to use even lower p$K_a$ boronic acid derivatives to avoid any pH dependence of the glucose response within the physiologic pH range.

We have continued to optimize the performance of our photonic crystal glucose-sensing materials for use in determining glucose in tear fluid. We report here measurements of the glucose response of our sensors in aqueous solutions containing the known components of tear fluid. We also explored the use of our novel 4-amino-3-fluorophenylboronic acid (AFBA)[4] derivative (p$K_a$ = 7.8) *(8)*

[4] Nonstandard abbreviations: AFBA, 4-amino-3-fluorophenylboronic acid; CFBA, 4-carboxy-3-fluorophenylboronic acid; DEAP, 2,2-diethoxyacetophenone; AA, acrylamide; bisAA, *N,N'*-methylenebisacrylamide; PEG, poly(ethylene glycol); EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; DMSO, dimethyl sulfoxide; and PCCA, polymerized crystalline colloidal array.

and examined the use of a 4-carboxy-3-fluorophenylboronic acid (CFBA) derivative. We also report here the first measurements of the glucose association constants for these boronic acid derivatives (Scheme 1).

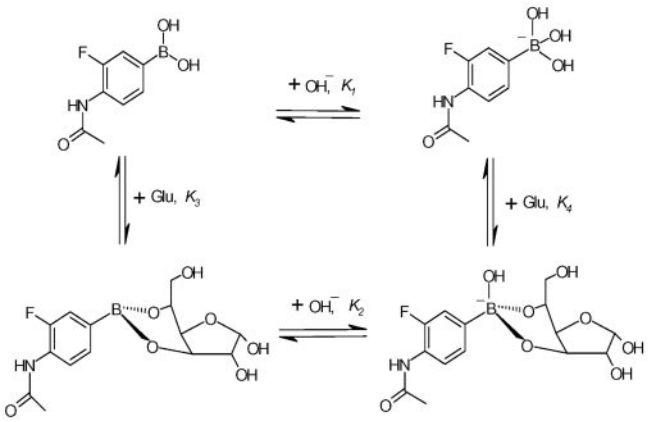
## Materials and Methods

β-D-(+)-Glucose, D-(+)-mannose, D-(−)-fructose, and D-(+)-galactose were purchased from Sigma and used as received. Glycylglycine hydrochloride (Sigma), sodium chloride (JT Baker), sodium bicarbonate (JT Baker), potassium chloride (Merck), urea (Merck), ammonium chloride (JT Baker), vitamin C (Aldrich), citric acid (Aldrich), lactic acid sodium salt (Fluka), pyruvic acid sodium salt (Sigma-Aldrich), chicken egg albumin (Sigma-Aldrich), bovine γ-globulins from Cohn fraction II and III (Sigma), chicken egg white lysozyme (Sigma-Aldrich), 2,2-diethoxyacetophenone (DEAP; Acros Organics), acrylamide (AA; Sigma), *N,N′*-methylenebisacrylamide (bisAA; Sigma), poly(ethylene glycol), monomethacrylate 400 (PEG; Polysciences, Inc.), HCl (JT Baker), NaOH (JT Baker), *N,N,N′,N′*-tetramethylethylenediamine (Aldrich), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC; Aldrich), CFBA (Ryscor Science), ethylene diamine (Fisher), dimethyl sulfoxide (DMSO; Fisher), 4-bromo-2-fluoro aniline (Acros), trimethylborate (Aldrich), *tert*-butyl lithium (1.7 mol/L in pentane; Aldrich), acetyl chloride (Aldrich), triethylamine (Aldrich), trimethoxyborate (Aldrich), and dichloromethane (Aldrich) were used as received.

Diffraction from the polymerized crystalline colloidal array (PCCA) was measured by a Spectral Instruments SI 400 diode array spectrometer. Ultraviolet spectra were measured with a Perkin-Elmer Lambda 9 and a Cary 500 Varian UV/Vis/NIR spectrometer.

### PREPARATION OF CCAs

Highly charged monodisperse polystyrene colloids were prepared by emulsion polymerization as described elsewhere *(30)*. We used either ~120 g/L suspensions of 154-nm polystyrene colloidal particles with a polydispersity of 5% or an ~50 g/L suspension of 95-nm particles with a polydispersity of 7% (QELS measurements). The suspensions were cleaned by dialysis against deionized water (17.5 MΩ/cm; Barnstead Nanopure Water Purification System) and by shaking with ion-exchange resin. The suspensions became iridescent as a result of Bragg diffraction from the CCA on shaking with ion-exchange resin. Each particle may possess as many as 60 000 strong acid groups *(30)*.




Scheme 1. Equilibria for 4-acetamido-3-fluorophenylboronic acid glucose binding.

### PREPARATION OF PCCAs

The PCCAs were synthesized by free radical solution polymerization using DEAP as a photoinitiator. A typical recipe (recipe A) used 100 mg (1.4 mmol) of AA, 2.5 mg (16 μmol) of bisAA, 0.02 g of PEG, 3 g of the CCA suspension of 154-nm diameter particles in deionized water, and ~50 mg of ion-exchange resin. This produces a sensing material with higher sensitivity because of a decrease in the polymer hydrogel concentration from that reported previously *(8)*. This polymerization mixture was shaken for 10–15 min, at which time 7 μL of a 100 g/L solution of DEAP in DMSO (3.4 μmol of DEAP) was added to the AA-PEG-bisAA-CCA suspension. The solution was shaken for an additional 10 min and then centrifuged for 30 s to precipitate the ion-exchange resin particles. This dispersion was injected into a cell consisting of two clean quartz discs separated by a 125-μm Parafilm spacer.

In the second recipe (recipe B), we used 100 mg of AA, 1.5 mg of bisAA, 0.02 g of PEG, and 3 g of the CCA suspension of 95-nm diameter particles. This recipe further decreased the cross-linker content and used a smaller particle size, which increased the sensitivity compared with recipe A.

Photopolymerization was performed with ultraviolet mercury lamps [Black Ray (365 nm)] for 40–60 min. The cells were opened, and the PCCAs were washed overnight with distilled water.

### CHEMICAL MODIFICATION OF HYDROGEL BACKBONE

We used two routes to modify the hydrogel backbone. We either hydrolyzed the amide groups to carboxylates or converted them to amines through aminoethylation.

*Hydrolysis route.* The PCCA hydrogel backbone was hydrolyzed in a solution containing 0.1 mol/L NaOH and 100 mL/L *N,N,N′,N′*-tetramethylethylenediamine for 3 h to convert amide groups to carboxylates. The hydrolyzed PCCAs were washed extensively and then immersed in a solution containing 25 mmol/L EDC and 25 mmol/L AFBA, pH 3, for ~3 h to obtain the AFBA-AA-PEG PCCAs. These PCCAs were repeatedly washed with 2 mmol/L glycylglycine–150 mmol/L NaCl (pH 7.4) buffer.

Because of the carboxylate charge, the washed hydrolyzed gels swelled extensively in water and diffracted in the infrared region. The gel diffraction after AFBA attachment returned almost to the visible spectral wavelength

characteristic of the original nonhydrolyzed PCCA, indicating that most of the carboxyl groups linked to the boronic acid derivatives. Atomic emission determination of the boron content (Desert Analytics Co.) indicated that we incorporated ~35 mmol/L AFBA.

*Aminoethylation route.* We developed an aminoethylation route (Scheme 2) for our PCCA photonic materials that preserves the CCA ordering and diffraction.

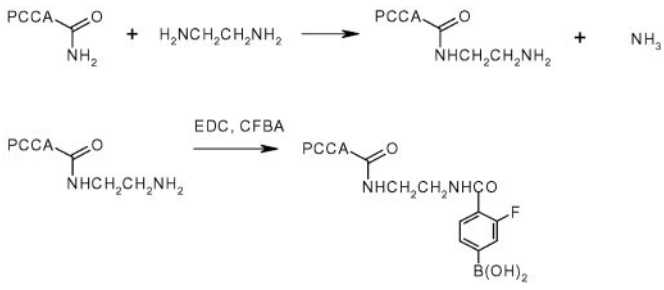
We used aminoethylation as described by Inman and Dintzis *(31)*. The AA-PEG PCCA on a quartz plate was transferred stepwise from pure water to DMSO by 200 mL/L incremental increases in the relative DMSO concentration. The PCCA was kept at each DMSO–water concentration for 30 min. After achieving a pure DMSO medium, the gel was transferred to 200 mL of a 100 mL/L ethylenediamine solution in DMSO and heated to 90 °C for 20 h in a round-bottom jacketed reaction vessel, which was sealed under nitrogen to avoid hydrolysis of amide groups (Scheme 2). After cooling, the hydrogel was repeatedly washed with DMSO and then coupled to CFBA through EDC coupling for 48 h (Scheme 2). We typically used 10–25 mL of 70 mmol/L EDC and 70 mmol/L CFBA in DMSO. After washing out the coupling solution with DMSO, we transferred the AA-PEG-CFBA PCCA from pure DMSO to a solution of 2 mmol/L glycylglycine–150 mmol/L NaCl (pH 7.4) through 200 mL/L incremental steps of DMSO–buffered saline.

Atomic emission determination of the boron content (Desert Analytics Co.) indicated that this procedure incorporated ~22 mmol/L CFBA.

### Chemical modification and synthesis of boronic acid derivatives for ultraviolet-visible titration

Ultraviolet-visible titration data were used to determine the p$K_a$ values and the glucose-binding constants for these boronic acid derivatives. Because these derivatives were attached to the hydrogel through an amide linkage, we estimated the linked derivative p$K_a$ by measuring the p$K_a$ of 4-acetamido-3-fluorophenylboronic acid, which we prepared from 4-bromo-2-fluoroaniline. We converted the amine group to its acetamido derivative by treating 4-bromo-2-fluoroaniline with acetyl chloride in dichloromethane at 0 °C in the presence of triethylamine *(32)*. In the second step we prepared the corresponding boronic acid derivative by trimethoxyborate lithium halide exchange followed by hydrolysis *(26)*.

3-Fluoro-4-*N*-methylcarboxamide phenylboronic acid was prepared from 4-carboxy-3-fluorophenylboronic acid as described previously *(33)*.




Scheme 2. Aminoethylation route for chemical modification of the PCCA polyacrylamide Hydrogel backbone.

## Results and Discussion

Our glucose-sensing hydrogel senses glucose through the formation of a supramolecular complex *(8)* between glucose, two boronates, two $Na^+$ ions, and PEG (Fig. 3). This glucose cross-link acts an additional hydrogel cross-link. The PEG localizes $Na^+$ close to the two boronates to form ion pairs to electrostatically stabilize the dianion complex by minimizing the electrostatic repulsion between the two boronates. These glucose-induced hydrogel cross-links increase the elastic-restoring force and shrink the hydrogel volume, which blueshifts the diffraction.

### Binding constants and p$K_a$

We used ultraviolet-visible absorbance titrations *(7)* to determine the p$K_a$ values and the glucose equilibrium binding constants of the amidated derivatives of the boronic acid species bound to the hydrogel. These were excellent models because the boronic acid derivatives attach to the hydrogel through amide linkages. We determined that 4-acetamido-3-fluorophenylboronic acid has a mean (SD) p$K_a$ of 7.81 (0.16), whereas 3-fluoro-4-*N*-methylcarboxamide phenylboronic acid has a p$K_a$ of 7.28 (0.04).

The glucose concentration dependence of the apparent p$K_a$ value for these boronic acid derivatives is shown in Fig. 4. The apparent p$K_a$ decreases with increasing glucose concentration because of the decreased p$K_a$ of the glucose-bound boronate complex *(23, 27, 33)* (Scheme 1). For example, at 1 mol/L glucose, the 4-acetamido-3-fluorophenylboronic acid derivative shows an apparent p$K_a$ of 5.8, whereas the 3-fluoro-4-*N*-methylcarboxamide phenylboronic acid derivative shows a p$K_a$ of 5.15. The

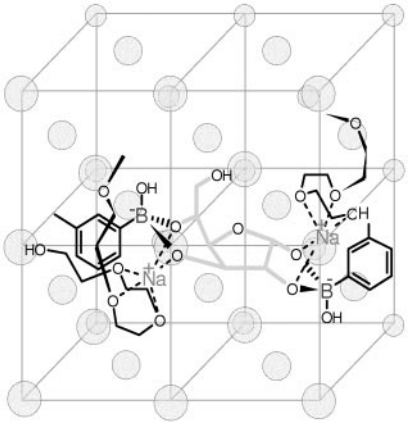



Fig. 3. Bis-bidentate complex formation between glucose (in furanose form) and two boronates stabilized by PEG-$Na^+$ complex.

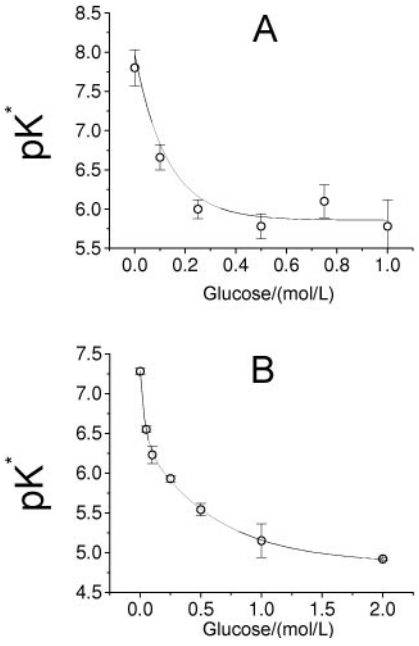



Fig. 4. Effect of glucose concentration on the apparent p$K_a$ values for hydroxylation of 4-acetamido-3-fluorophenylboronic acid (*A*) and 3-fluoro-4-*N*-methylcarboxamide phenylboronic acid (*B*).

The *solid lines* are theoretical best fit of the data as described previously *(8)*.

solid lines in Fig. 4 are the best fits of the data to theory as described previously *(8)*.

The equilibrium binding constants (Scheme 1) are summarized in Table 1. The constant $K_4$ describing the affinity for glucose of the anionic boronate form of 4-acetamido-3-fluorophenylboronic acid is 370 (40) L/mol, whereas for 3-fluoro-4-*N*-methylcarboxamide phenylboronic acid it is 284 (30) L/mol. These values are higher than the $K_4$ (110 L/mol) reported by Lorand and Edwards *(27)* many decades ago for 3-aminophenylboronic acid. The glucose-binding affinity is a major factor limiting the sensitivity of our sensors to glucose. These results suggest that these boronic acid derivatives can increase our sensitivity approximately threefold compared with 3-aminophenylboronic acid for pH values at which the boronate species are present.

We also determined the binding constant of glucose to the neutral boronic acid derivatives. $K_3$ was $<3.7$ for 3-fluoro-4-acetamide phenylboronic acid, whereas it was $<1.2$ for 3-fluoro-4-*N*-methylcarboxamide phenylboronic acid. Thus, even neutral boronic acids bind to glucose. Determining $K_3$ is challenging because of its low value. Our $K_3$ measurement is one of the first *(34)* for glucose binding.

### PCCA GLUCOSE RECOGNITION AND SIGNALING

We are designing our glucose-sensing material to determine glucose in tear fluid. The expected glucose concentrations in tear fluid should be ~400 μmol/L *(35)*, more than 10-fold lower than the concentration of blood glucose (4–6 mmol/L) *(36)*. Daum and Hill *(37)* reported that the mean tear-fluid glucose concentration in healthy humans is 420 μmol/L (7.5 mg/dL), based on an average of 875 measurements.

We require that the diffraction of our glucose-sensing material be sufficient to allow easy differentiation between normoglycemic and hypo- and hyperglycemic concentrations. Fig. 5 shows the dependence on glucose concentration of diffraction of the AFBA-AA-PEG PCCA photonic crystal in 2 mmol/L glycylglycine buffer at pH 7.4 in the presence of 150 mmol/L NaCl, a concentration similar to that in body fluids. The spectral peaks originate from diffraction of normally incident light by the 111 planes of the embedded face-centered cubic CCA. We dramatically improved the sensitivity and working spectral window of our sensing motif compared with the response of our earlier amino-fluorophenylboronic acid PCCA glucose sensor *(8)* by optimizing the PCCA composition, mainly by decreasing the cross-link density; we use an AA-to-bisAA ratio of 40:1 compared with 16:1, which we used in previous studies. In addition, we found that boronic acid coupling was optimized at pH 3.0.

This composition change increased both the sensor sensitivity and its response window. For example, our previous photonic glucose sensor *(8)*, which contained AFBA, had a working window of 75 nm (0–20 mmol/L), whereas the photonic glucose sensor presented here has a working window 180 nm with a limit of detection (blank plus 3 SD of a blank; n = 10) as low as 10 μmol/L glucose.

In the absence of glucose, the sensor diffracts 640 nm red light (Fig. 5). The diffraction blueshifts with increasing glucose concentrations. For example, the sensor diffracts 616 nm reddish-orange light at 100 μmol/L glucose, 603 nm orange light at 200 μmol/L, and 581 nm greenish-yellow light at 500 μmol/L. The diffraction blueshift continues with higher glucose concentrations up to 20 mmol/L, where the sensor diffracts 454 nm violet light.

Further increases in glucose concentration above 20 mmol/L cause diffraction redshifts. For example, at 40 mmol/L glucose, the diffraction redshifts to 458 nm (Fig. 5). The color changes are bright and visually evident, as seen in the top photograph in Fig. 5. Obviously, the diffracted color can be easily viewed and used for glucose determination by use of a calibration color chart. The

**Table 1. Equilibria constants associated with boronic acids glucose binding.**

| Boronic acid | $K_1$,[a] L/mol | $K_2$, L/mol | $K_3$, L/mol | $K_4$, L/mol |
|---|---|---|---|---|
| 3-Fluoro-4-*N*-methylcarboxyamide phenylboronic acid | $5.2 \times 10^6$ | $1.2 \times 10^9$ | <1.2 | 284 |
| 3-Fluoro-4-acetamide phenylboronic acid | $1.6 \times 10^6$ | $1.6 \times 10^8$ | <3.7 | 370 |

[a] Mean (SD) p$K_a$ = 7.28 (0.04) for 3-fluoro-4-*N*-methylcarboxyamide phenylboronic acid and 7.8 (0.1) for 3-fluoro-4-acetamide phenylboronic acid.

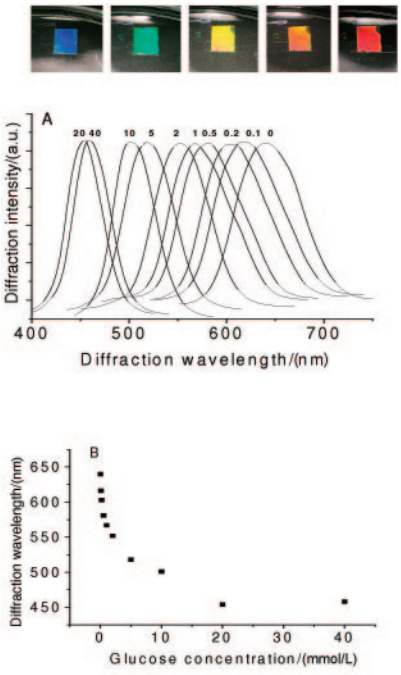



Fig. 5. Effect of glucose concentration on diffraction of the AFBA-AA-PEG PCCA sensor (recipe A) in 2 mmol/L glycylglycine (pH 7.4)–150 mmol/L NaCl.

(*Top*), diffraction color changes from red to blue with increasing glucose concentration. (*A*), dependence of diffraction wavelength on glucose concentration. The diffraction peaks are labeled with the corresponding glucose concentration (mmol/L). *a.u.*, absorbance unit(s). (*B*), dependence of diffraction peak maxima on glucose concentration.

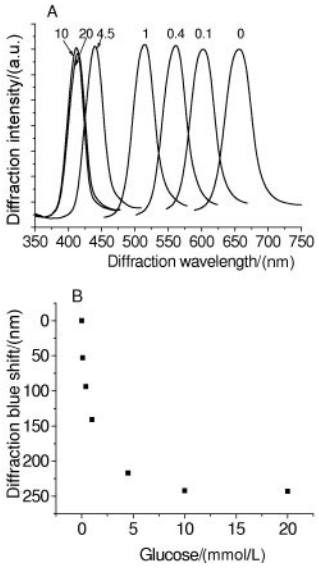



Fig. 6. Effect of glucose concentration on diffraction of the AFBA-AA-PEG PCCA sensor (recipe B) in 2 mmol/L glycylglycine (pH 7.4)–150 mmol/L NaCl.

(*A*), diffraction peaks are labeled with the corresponding glucose concentration (mmol/L). *a.u.*, absorbance unit(s). (*B*), dependence of diffraction peak shift on glucose concentration.

CFBA-AA-PEG glucose sensor demonstrated a response to glucose comparable to that of the AFBA-AA-PEG sensor.

The optimum PCCA composition for a glucose sensor for tear fluid uses recipe B (Fig. 6). It has a spectral window of 250 nm, which spans the entire visible spectral window. In the absence of glucose, the sensor diffracts a 658 nm red light. At 100 $\mu$mol/L glucose, it diffracts 615 nm orange-reddish light; at 400 $\mu$mol/L, it diffracts 569 nm yellow-green light; at 1 mmol/L, it diffracts 523 nm green light; at 4.5 mmol/L, it diffracts 449 nm blue light; and at 10 mmol/L glucose, it diffracts violet 424 nm light. The detection limit (blank plus 3 SD of a blank; n = 10) for this material is ~1 $\mu$mol/L glucose.

The PCCA photonic glucose sensors demonstrate selectivity for glucose. Glucose is rather unique in that it is able to form bis-bidentate complexes with boronic acid derivatives *(38–40)* because of its two sets of *cis*-diols. Other physiologically important sugars, such as fructose, galactose, and mannose, are unable to form bis-bidentate complexes at neutral pH; they form mono-bisdentate complexes to boronic acids, producing diffraction red-shifts as demonstrated previously *(8)*.

Takeuchi et al. *(41)* also observed bisboronate-glucose complexes in a bisboronic acid-ferrocene derivative. They also observed that D-galactose and D-mannose did not form the bis-bidentate complexes. In D-glucose, the 2- and 4-hydroxy groups adopt the same configuration and thus both bind to the boronate, whereas the D-mannose and D-galactose hydroxyl groups adopt opposing configurations. Shiomi et al. *(39)* concluded that this leads to steric distortions that hamper the formation of D-galactose and D-mannose bis-bidentate complexes. As shown in Fig. 7, a glucose cross-linking-induced blueshift dominates the tear-fluid sensor response. The concentrations of glucose and galactose expected in tear fluid are 400 and 40 $\mu$mol/L, respectively *(35)*; thus, glucose is a dominant tear-fluid carbohydrate.

We also studied the dependence of the PCCA glucose sensor response to glucose in a synthetic tear-fluid solution formulated from the Geigy Scientific Tables *(42)*. The

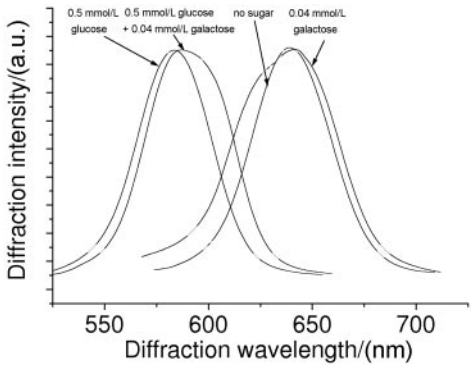



Fig. 7. Effect of glucose concentration on diffraction of the AFBA-AA-PEG PCCA sensor (recipe A) in 2 mmol/L glycylglycine (pH 7.4)–150 mmol/L NaCl in the presence of 0.04 mmol/L galactose, 0.5 mmol/L glucose, or 0.5 mmol/L glucose plus 0.04 mmol/L galactose.

*a.u.*, absorbance unit(s).

**Table 2. Composition of artificial tear fluid (pH 7.4) formulated according to Geigy formula *(42)*.**

| Component | Concentration |
|---|---|
| Sodium chloride | 100 mmol/L |
| Sodium bicarbonate | 26 mmol/L |
| Potassium chloride | 16 mmol/L |
| Urea | 5 mmol/L |
| Ammonia chloride | 3 mmol/L |
| Lactic acid | 2.5 mmol/L |
| Pyruvic acid | 0.2 mmol/L |
| Citric acid | 31 μmol/L |
| Vitamin C | 8 μmol/L |
| Albumins | 3.94 g/L |
| γ-Globulins | 2.75 g/L |
| Lysozyme | 1.7 g/L |

artificial tear-fluid composition is given in Table 2. The pH of the artificial tear fluid was adjusted with diluted HCl to a set value of 7.4. Shown in Fig. 8 is a comparison of the response of the PCCA glucose sensor to glucose in artificial tear fluid and buffered saline [2 mmol/L glycylglycine (pH 7.4), 150 mmol/L NaCl]. We observed no interference from the components of the tear fluid with our glucose sensing. A major component of tear fluid that might interfere with glucose sensing are proteins. Our results demonstrate identical diffraction blueshift for glucose in the presence or absence of proteins is practically the same. This result demonstrates the feasibility of our approach for use in sensing glucose in tear fluid.

We are also aware that the pH of tear fluid can decrease during the night time when the eyelids are closed and increase during the day *(43–45)*. This pH shift occurs because, when the eyelids are open and tear fluid contacts the atmosphere, carbon dioxide can be released from the tear fluid, decreasing the pH. The pH ranges between 7.1 and 7.8 among individuals at different times

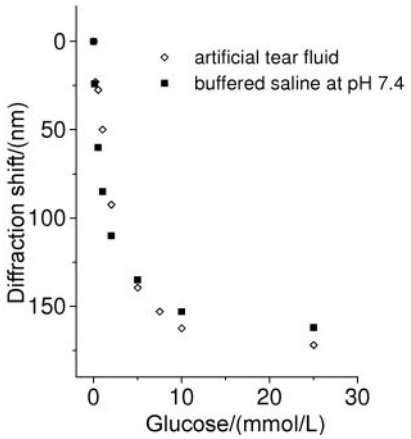



Fig. 8. Effect of glucose concentration on the diffraction peak shift of the PCCA (recipe A) in artificial tear fluid and 2 mmol/L glycylglycine (pH 7.4)–150 mmol/L NaCl.

In the absence of glucose, the sensor diffracts 640 nm light in buffered saline and 646 nm light in artificial tear fluid.

of the day *(46)*. According to van Haeringen *(45)*, the higher values reported in the literature originate from shortcomings in technique resulting from carbon dioxide loss during sampling. Because boronic acid glucose sensing is pH-dependent, we expect to further refine our glucose-sensing material, using boronic acid derivatives with $pK_a$ values lower than 7.0. These derivatives are currently under study in our group and will be the subject of a separate report.

In this report, we demonstrated the high potential of the photonic glucose-sensing material to sense and target glucose at physiologic pH and the ionic strength typical of bodily fluids; it thus is suitable for in vivo glucose monitoring. The prototype ocular photonic glucose sensor described here is an inexpensive, reagentless, and attractive alternative to the existing methods of glucose monitoring in patients with diabetes.

In conclusion, we demonstrate an improved photonic crystal glucose sensor consisting of a polyacrylamide-PEG polymer network with an embedded CCA that reports on glucose concentrations at physiologic ionic strengths and at pH 7.4. We used new boronic acid derivatives, such as AFBA and CFBA, as recognition elements to bind and sense glucose at physiologic pH. Glucose binding to boronates occurs through formation of a bis-bidentate cross-link, which shrinks the hydrogel volume. These volume changes in turn cause the diffraction from the embedded CCA to blueshift in proportion to the glucose bound. The color changes, perceived visually without any instrumentation, shift across the visible spectral region, from red to blue over physiologically relevant glucose concentrations. This material responds to glucose in artificial tear fluid without interference from the major components of tear fluid. Thus, our photonic glucose-sensing materials may be suitable for fabrication of diagnostic contact lenses or ocular inserts to monitor the glucose concentration in tear fluid of patients with diabetes mellitus.

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