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Fast Responsive Crystalline Colloidal Array Photonic Crystal Glucose Sensors

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We developed new photonic crystal polymerized crystalline colloidal array (PCCA) glucose sensing materials, which operate on the basis of formation of cross-links in the hydrogel. These materials are composed of hydrogels that embed an array of ~100-nm-diameter monodisperse polystyrene colloids that Bragg diffract light in the visible spectral region. The hydrogels change volume as the glucose concentration varies. This changes the lattice spacing, which changes the wavelength of the diffracted light. In contrast to our previous glucose sensing photonic crystal materials, we no longer require Na^+ chelating agents. These photonic crystal materials are being designed for use in glucose sensing contact lens for people with diabetes mellitus. We describe methods to speed up the response kinetics of these PCCA sensing materials. Rapid-response kinetics is achieved by controlling the elasticity and the hydrophilic−hydrophobic balance of the hydrogel system. A more hydrophobic hydrogel composition is obtained by copolymerizing *n*-hexylacrylate into an acrylamide−bisacrylamide hydrogel. The response rate significantly increases to where it fully responds within 90 s to the average glucose concentrations found in blood (5 mM) and within 300 s to the average glucose concentrations found in tear fluid (0.15 mM). We find unusual temperature-dependent kinetics, which derive from glucose mutarotation in solution. It is shown that α-D-glucose is the glucose anomer binding to the boronic acid derivative. Care must be taken in any glucose determination to ensure that the glucose mutarotation equilibrium has been established. We have demonstrated that the sensor is responsive to ~0.15 mM glucose concentrations in artificial tear fluid solution.

Noninvasive glucose sensing methodologies would greatly benefit patients with diabetes mellitus. To aid in this effort, we recently developed a polymerized crystalline colloidal array (PCCA) glucose sensing material,[1] which can be used as the sensing element of a minimally invasive glucose sensing contact lens to measure tear fluid glucose. Our technology[2−4] is based on a glucose-responsive hydrogel, containing fluoroaminophenylboronic acid as the molecular recognition agent, as well as a photonic crystal PCCA to diffract visible wavelength light. The system changes its diffraction wavelength in response to changes in the concentration of glucose at levels as low as 1 μM.

This photonic crystal chemical sensing technology can be modified to sense numerous species, and the materials can be tailored for numerous potential applications. Many applications, including glucose sensing are time sensitive and require that the sensing occur within time periods over which concentration changes are physiologically important. For example, for diabetics it is desirable to have response times shorter than the time scale of physiological glucose excursions, to permit continuous glucose monitoring.

The work reported here has further developed our PCCA glucose sensing materials and has sped up the response time of our glucose sensors by systematically examining the dependence of the sensing kinetics on the hydrogel composition and the rates of glucose mutatrotation. Surprisingly little information is available on the kinetics of hydrogel volume changes.

Most of the information on hydrogel volume change kinetics derives from studies of the temperature-driven hydrogel volume phase transitions of poly(*N*-isopropylacrylamide) (p-NIPAM) hydrogels.[5,6] This polymer undergoes a volume phase transition from a highly swollen state in water, below room temperature, to a shrunken, almost pure polymer state at T ~32 °C. This process is fully reversible, and volume changes as large as 30-fold are common. The slow response rates of minutes to hours of these systems have been attributed to the slow transport properties of the polymer network, limiting solvent diffusion constants, and limited flow inside the hydrogel network. It has also been demonstrated that hydrogel volume phase transitions can also be impeded and slowed by formation of skin layers[7] at the hydrogel surface due to collapse of the hydrogel at the interface with the solution. This skin limits flow in to and out of the hydrogel.

The volume phase transition response times of p-NIPAM hydrogels have been sped up by, for example, reducing the hydrogel size,[8,9] thus, allowing faster equilibration due to the reduction of the volume of transported solvent and the length over which it is exchanged. For example, we demonstrated that 100-

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nm-diameter colloidal particles of p-NIPAM show microsecond volume changes[10] Introducing pores into the hydrogel, to obtain micro-[11,12] or superporous[6] structures, can decrease the response time by orders of magnitude (from hours and days down to minutes) to reach maximum swelling and deswelling volumes. Tanaka and Fillimore have also shown[8] that the hydrogel kinetics is proportional to the diffusion coefficient of the gel network, defined by $D = E/f$, where E is the longitudinal bulk modulus of the network and f is the coefficient of friction between the network and the gel fluid.

Porous structures increase the surface-to-volume ratio, thus allowing faster diffusion into the hydrogel. These pores were mainly produced by phase separation methods[13] using a mixed solvent system that allows full expansion of the polymer chain during polymerization. The fast deswelling characteristics are obtained by polymerizing in the presence of pore-forming molecules[14] such as poly(ethylene glycol) (microporous) or by producing gas bubbles during polymerization using a chemical reaction[15] (macro- or superporous). Performing the polymerization at low temperatures[16] (below the solvent freezing point) or by a freeze-drying method[17] also yields highly porous, fast-responding hydrogels.

Another approach introduces graft polymers onto the hydrogel backbone[18,19] to allow rapid transport through the hydrogel. In this approach, hydrophobic modifications of the hydrogel network decrease the response times due to the "rapid dehydration of the relatively mobile grafted chains that serve as nucleation sites for the aggregation of the cross linked chains".

Our hydrogel PCCA sensor volume response will have richer hydrogel volume phase transition behaviors than do simple hydrogels. Our glucose sensing volume response derives from the glucose cross-linking of boronic acid groups covalently bound to the hydrogel. In addition, the resulting hydrogel volume response could also be limited by glucose mutarotation rates.

D-Glucose, the common optically active isomer,[34] occurs in solution in an equilibrium between four different isomers: α- and β-glucofuranose and α- and β-glucopyranose anomers. These species interconvert through the linear aldehydoglucose form in a process known as mutarotation. At equilibrium,[20] ~60.9% occurs in the more stable β-pyranose form while 38.8% occurs in the α-pyranose form. Only small fractions occur in the furanose forms (~0.15% of each).

Several groups have investigated the possible structures formed between glucose and phenylboronic acid derivatives in aqueous solutions.[21−23] Although, glucose initially appears to complex in the pyranose form, it quickly mutarotates to form the more stable furanose ligand. This is clear from the Norrild and Eggert[22,23] investigation of the binding of a boronic acid derivative using ^{1}H and ^{13}C NMR, which found that glucose binding shows a strong preference for the α-glucofuranose form in water. Cooper and James[24] showed that for most cases the changes in fluorescence for fluorescent boronic acid derivatives differ between saccharides that cannot equilibrate between the pyranose and furanose forms (D-maltose and D-leucrose) compared to those like D-glucose that can. Their data suggest that for D-glucose the fluorescence increases because it binds in its α-glucofuranose form, which results in a stronger B−N interaction, which has a higher fluorescence quantum yield.[24]

In the work here, we investigated the kinetics of acrylamide−bisacrylamide hydrogel network volume phase transitions in a glucose sensing PCCA. We developed a new faster responding composition, where we copolymerize *n*-hexylacrylate into a acrylamide bisacrylamide hydrogel. We examined the impact of changing the cross-link density, the temperature, and the hydrogel hydrophobicity on the kinetics. We also examined the dependence of the response kinetics to equilibration of glucose anomers.

EXPERIMENTAL SECTION

Materials. D-(+)-Glucose (99.5%, Sigma), α-D-glucose (96%, Sigma), β-D(+)-glucose (97%, Sigma), glycylglycine hydrochloride (Gly-Gly, Sigma), NaCl (J.T. Baker), 2,2-diethoxyacetophenone (DEAP, 98%, Acros Organics), acrylamide (AA, 98%, Sigma), *N,N′*-methylenebisacrylamide (BisAA, 98%, Sigma), *n*-hexyl acrylate (98%, Polysciences Inc.), HCl (J.T. Baker), NaOH (J.T. Baker), dimethyl sulfoxide (DMSO, Fisher), *N,N,N′,N′*-tetramethylethylenediamine (TEMED, 98.5%, Sigma), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (98%, Sigma), and 5-amino-2-fluorophenylboronic acid (5A-2F-PBA, 98%, Asymchem) were used as received. Stock solutions of AA−BisAA (49:1, 29:1, 19:1, Sigma) were prepared by dissolving premixed powders (purchased from Sigma) in deionized water (Barnstead Nanopure Water Purification System).

Preparation of CCA. Highly charged monodisperse polystyrene colloids were prepared by emulsion polymerization as described elsewhere.[25] We used a suspension of 145-nm polystyrene colloidal particles (PS, ~8 wt %) with a polydispersity of ~5% (determined by DLS measurements). The suspensions were cleaned by dialysis against deionized water and by shaking with ion-exchange resin.

Preparation of PCCA. The PCCA was synthesized by free radical solution polymerization using DEAP as a photoinitiator. In a typical recipe, ~0.2 g of ion-exchange resin and ~2.8 g of the PS suspension were shaken together. The appropriate amounts of monomers (acrylamide and *n*-hexyl acrylate; Table 1) and cross-linker (bisacrylamide; Table 1) were added and shaken together for an additional 10 min. The total polymer

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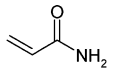
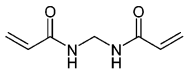
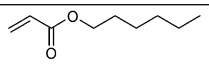
Table 1. Chemical Structures of the Monomers Copolymerized in the Hydrogels

Name	Symbol	Chemical structure
Acrylamide	AAm	
N,N′-Methylenebisacrylamide	Bis-AAm	
n-hexyl acrylate	n-HA	

content ranged from 3.8 to 8.8 wt % depending on the hydrogel prepared. Then, 40 μL of a 10% DEAP solution in DMSO was added, and the solution was further shaken for an additional 2 min and then centrifuged for 10 s to precipitate the ion-exchange resin particles. This dispersion was injected into a cell consisting of two clean quartz disks separated by a 125-μm spacer. Photopolymerization was performed with UV mercury lamps [Black Ray (365 nm)] for up to 120 min. The cells were opened, and the PCCA films were washed overnight with distilled water. The PCCA hydrogel backbone was hydrolyzed in a 10% solution of *N,N,N′,N′*-tetramethylethylenediamine containing 0.1 M NaOH for 1.5 h in order to convert amides to carboxylates. The hydrolyzed PCCA was washed extensively and then immersed in a solution containing 25 mM EDC and 25 mM 5A-2F-PBA at pH 3 for various times in order to attach the boronic acid derivatives to the polymer backbone. The PCCA were repeatedly washed with a solution of 5 mM Gly-Gly containing 150 mM NaCl (pH 7.4). By changing the coupling conditions, we increased the amount of incorporated boronic acid derivatives from 27 mM up to 200 mM (as determined by an ICP-AES analysis of boron content by Desert Analytics Lab, Tuscon, Az).

Diffusion Measurements. For the diffusion measurements, the PCCA hydrogel was polymerized within a 5-μm nylon mesh matrix (Small Parts Inc., Miami Lakes, Fl.). The nylon mesh containing the hydrogel was 250 μm thick (twice the thickness of our typical hydrogels). The diffusion constant through the PCCA inside the nylon mesh was determined by dialysis by placing the membrane between two dialysis cells. A 7-mL aliquot of 10 mM glucose solution was added to the reservoir cell, and 7 mL of pure water was added to the receiver cell. Small stirring bars were placed in both compartments for mixing. The 200-μL aliquots of the solution from the receiver cell (initially pure water) were withdrawn after 15, 30, 45, 60, 120, and 180 min and overnight. The same volume aliquots were removed from the glucose solution compartment. The glucose content was analyzed using a Radiometer ABL 700 Series blood gas analyzer.

The experimentally determined diffusion coefficient, D, was calculated using Fick's first law:

$$D = -J/(\Delta c/\Delta x)$$

where J is the flux, Δc is the concentration difference between the cells at the specific sampling time, and Δx is the thickness of the hydrogel.

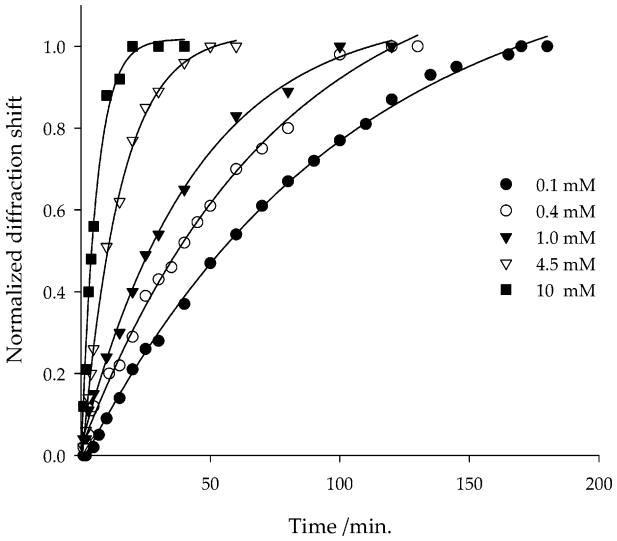


Figure 1. Response kinetics of the previous glucose sensor[1] to freshly prepared solutions of β-D-glucose in Gly-Gly buffered solution, pH 7.4, containing 150 mM NaCl. The different plots refer to 0.1 (●), 0.4 (○), 1.0 (▼), 4.5 (▽), and 10 mM (■) β-D-glucose (fit lines are guide to the eye).

Determination of pK_a Value. The pK_a value of 5A-2F-PBA was determined by UV−visible titration.

Diffraction Measurements. Diffraction from the PCCA was measured by an Ocean Optics USB-2000 fiber-optic spectrometer in reflectance mode. The diffraction measurements at 37 °C were performed in a covered thermostated water bath at a pH of 7.4.

RESULTS AND DISCUSSION

Our previously fabricated PCCA glucose sensor[1,4] utilized a supramolecular bisbidentate glucose−boronic acid complex where PEG or a crown ether group bound sodium ions to neutralize the boronate charges. This increased the association constant of the bidentate complex, which acted as a cross-link to shrink the hydrogel under these high ionic strength conditions. Thus, the hydrogel diffraction blue shifts in response to increasing glucose concentrations.

The response time of this sensor depends on the glucose concentration (Figure 1). For example, 90% of the maximal diffraction shift occurs within ~15 and ~90 min in response to the introduction of 10 and 1 mM β-D-glucose in a pH 7.4 aqueous solution containing 150 mM NaCl, respectively. The response time lengthens to multiple hours for a 0.1 mM β-D-glucose concentration, which is the relevant concentration of glucose in tear fluid.[35] These extremely slow response times of this glucose sensor clearly limits the potential utility of this sensing technology.

In the work here, we demonstrate a new PCCA glucose sensing material that does not require Na^+ chelating agents (PEG or crown ethers) to form glucose cross-links. We find that glucose−boronate bisbidentate cross-links will form in the presence of high concentrations of either phenylboronic acid bound to the hydrogel or the boronic acid 5A-2F-PBA derivative bound to the hydrogel. These high boronic acid loaded PCCA show diffraction blue shifts as the concentration of glucose increases in aqueous solutions containing 150 mM NaCl at pH values close to the pK_a of the boronic acid derivative.

The glucose response mechanism of these new glucose sensing PCCA clearly differs from PCCA sensors made with ~10-

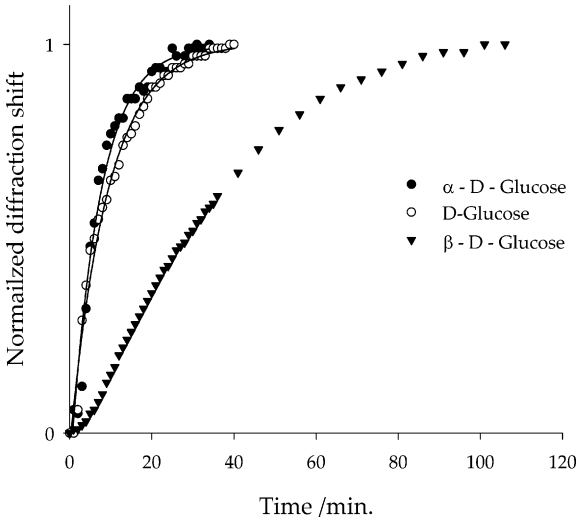


Figure 2. Response kinetics to a freshly prepared solution containing 1 mM α-D-glucose (◆), D-glucose (○), or β-D-glucose (▼) in Gly-Gly buffered solution, pH 7.4, containing 150 mM NaCl (fit lines are guide to the eye).

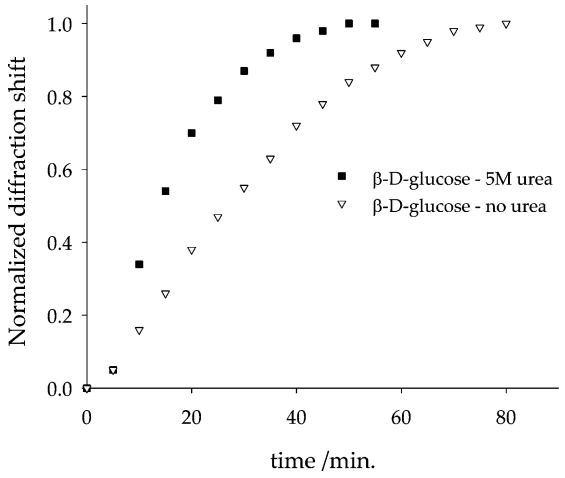


Figure 3. Response kinetics to a freshly prepared solution containing 1 mM β-D-glucose with 5 M urea (■) and without urea (▽) in Gly-Gly buffered solution, pH 7.4, with 150 mM NaCl.

fold lower concentrations of boronic acid derivatives, but without PEG or crown ethers.[32] PCCAs made with ~10-fold lower boronic acid derivative concentrations without PEG or crown ethers do not respond to glucose in high ionic strength solutions. In contrast, they show diffraction red shifts in response to glucose in low ionic strength solutions.

Our new glucose sensing material also has a response mechanism very different from that of the high polymer inverse opal PCCA of Braun et al.,[26] which also contain high boronic acid concentrations; their sensor red shifts in response to glucose in high ionic strength solutions. This suggests a mechanism that relies on a more favorable free energy of mixing upon glucose binding. We also examined the response of our sensor in the buffer system used by Braun et al. to rule out the possibility that the response difference was due to solution composition. Our sensor continues to blue shift in response to glucose in the presence of their buffer solution.

Glucose Mutarotation. To complicate any analysis, we find that the glucose mutarotation equilibration takes a significant time to occur[27] and that measurements made using fresh glucose solutions can yield very confusing results.

The glucose mutarotation process can be quite slow at pH 7 in the absence of catalysis. The slowness of the mutarotation process is evident from the fact that exposing our new sensors to freshly prepared α-D-glucose solutions gives 4-fold faster response kinetics than occurs in response to the same concentrations of β-D-glucose (Figure 2).

Obviously, the system has not come to equilibrium over the >1-h time frame of these measurements.[27] The faster response rate of the α-anomer compared to the β-anomer is expected from the results of both Norrild[22] and Shinkai,[21] who showed that glucose binds in the α-furanose or α-pyranose form(s). Figure 2 also shows that the response kinetics to a freshly prepared D-glucose solution is almost as fast as for the pure freshly prepared α-D-glucose. This indicates that this sample of D-glucose is primarily in the α-form.[28]

To understand the extent to which mutarotation controls the sensor response kinetics, we exposed our sensor to β-D-glucose and to solutions of D-glucose (primarily α-D-glucose) that contained high concentrations of urea. Panov and Sokolova[29] demonstrated that the rate of mutarotation increases in the presence of urea. Urea can hydrogen bond with water and the hydrogel since it can be both a proton donor and acceptor. Urea may also influence the response kinetics by breaking intrahydrogel hydrogen bonding, which could dramatically change the diffusion properties of the hydrogel chains relative to one another.

Soaking the sensor material in urea solutions causes the sensor to swell and red shift the diffraction wavelength 76 nm. Upon addition of 1 mM β-D-glucose (Figure 3), we see a 37% reduction in the response time in the presence of 5 M urea compared to β-D-glucose in the absence of urea. This result is close to that expected from Panov and Sokolova[29] for the increase in the mutarotation rate in the presence of 5 M urea (36% increase at 298 K).

These results demonstrates that the slow response time in the presence of β-D-glucose results from the time required for mutarotation to create the α-anomers; the formation time of the 5A-2F-PBA α-anomer is faster than the mutarotation time. In contrast, the increased urea-induced mutarotation rate for a freshly prepared 1 mM D-glucose solution (which is mainly the α-anomer) cannot significantly impact the sensor response kinetics, since binding of the α-anomer is so fast. Thus, we have established the speed limit associated with mutarotation and for all experiments except those noted have utilized glucose solutions containing the fast binding anomers.

We have further examined the rate of glucose complexation to boronates by studying the absorbance spectral kinetics after mixing boronic acid derivatives with the different freshly prepared glucose solutions. A UV−visible spectrum of a freshly prepared 5A-2F-PBA solution shows a strong absorbance at $\lambda = 298$ nm evident from the $\pi-\pi^*$ transition of the aminofluoro-substituted

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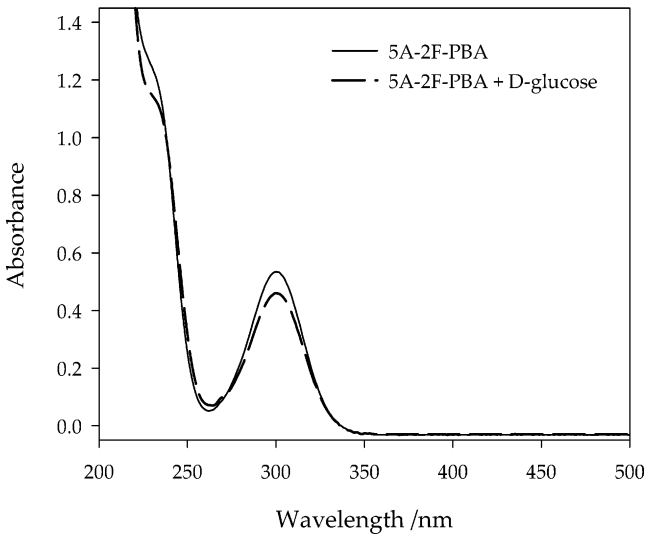

Figure 4. UV−visible spectrum of 5A-2F-PBA (10 mM) in Gly-Gly buffer, pH 7.4 (—), and after equilibration with D-glucose (– –). Path length, 1 mm.

phenyl ring (Figure 4). The peak absorbance undergoes complex kinetics upon addition of glucose.

Figure 5 shows the change in absorbance at 304 nm after adding α- and β-D-glucose to 5A-2F-PBA solutions at 22 and 37 °C. At 22 °C, addition of a 10-fold excess of β-D-glucose results in a fast absorption decrease, which is followed by an absorption increase that saturates within 3120 ± 10 s at 0.2 absorbance unit below the initial absorption value (note the 2-mm path length in Figure 5). At 37 ° C, we see ~3−4-fold faster kinetics than at 22 °C. In addition, the kinetics saturate at a 0.1 absorption decrease compared to that in the absence of glucose.

At both temperatures, the α-D-glucose spectral changes are ~3−4-fold faster than for β-D-glucose, saturating in ~1560 ± 10 s at 22 ° C and in ~540 ± 10 s at 37 ° C. We also performed experiments with glucose solutions after full mutarotation equilibrium was established (overnight aging at room temperature). These solutions exhibited intermediate kinetics at room temperature and fast kinetics at 37 °C, as with the α-anomer. All the results are summarized in Table 2.

Performing the experiment with D-leucrose (a sugar that is always in the pyranose form, Table 3) at 22 °C results in an ultrafast change in the absorbance of <10 s, much faster than for α-D-glucose. This result indicates that the preferred boronic acid binding anomer is the α-furanose form as previously argued by Norrild[22,23] and James.[24] We assume that the complex absorption kinetics of Figure 5 derive from initial binding of the α-pyranose form, followed by mutarotation into the preferred α-furanose form.

Furthermore, the fact that there is no reincrease in absorption at 298 nm, in the case of D-leucrose, indicates that the reincrease monitored for α- and β-glucose may be attributed to the glucose mutarotation equilibration after binding.

The UV−visible absorption kinetics are similar to those found for our crystalline colloidal array photonic crystal sensors to the freshly prepared glucose solutions. As described earlier the room temperature sensor response to a freshly prepared, 1 mM, α-D-glucose solution saturates in ~1260 ± 10 s, while the response to the β-anomer is extremely slow and takes ~5400 ± 10 s. As shown below, our sensor response also dramatically speeds up with increasing temperature; the sensor responds ~ 5 times faster at 37 °C than at 22 °C.

We also measured the response kinetics of our glucose sensor to buffered solutions of 1 mM D-leucrose and found response kinetics of ~1440 ± 10 s (~10-nm red shift of the diffraction wavelength) at 22 °C. As mentioned above, D-leucrose has two pyranose rings similar to those of the α-glucopyranose, but it cannot mutarotate into a furanose form. These results demonstrate that the response time of the hydrogel to D-leucrose is comparable to the response to a freshly prepared α-D-glucose solution. As shown below, this slow response kinetics is not limited by mutarotation but limited by the hydrogel kinetics.

Diffusion Measurements. We also tested the hypothesis that glucose diffusion through the hydrogel limits the response kinetics. The diffusion constant of glucose[30] at room temperature in water is 5.2×10^{-6} cm^2/s. Our PCCA hydrogel is mainly water, and we expect relatively free diffusion of glucose in PCCA with a diffusion constant slightly smaller than in water. The 100-μm thickness of the PCCA should give rise to ~10-s characteristic times for glucose equilibration into the sensor.

Before measuring diffusion through the PCCA, we performed several control experiments to eliminate the possibilities that the Nylon mesh or details of the hydrogel preparation could slow diffusion. We first examined the impact of a 100-μm-thick sheet of the 5-μm-pore size nylon mesh membrane on glucose diffusion. In this experiment, 7 mL of a 10 mM aqueous glucose solution was placed in the first dialysis cell, and 7 mL of water was placed in the other dialysis cell. Within 30 min, the initial 10 mM glucose solution concentration decreased to 9.2 mM glucose, while the receiver glucose concentration increased to 2.4 mM. The calculated glucose diffusion coefficient from this measurement is 3.4 $\times 10^{-6}$ cm^2/s, which is close to the glucose in pure water diffusion coefficient of 5.2×10^{-6} cm^2/s. Thus, this 5-μm nylon mesh only slows down the diffusion of glucose to 65% of it value in pure water.

We then examined glucose diffusion for PCCA hydrogels and for hydrogels without CCA polymerized within this 5-μm nylon mesh. We also eliminated the possibility that Sigmacote treatment of our quartz cell surfaces caused formation of a skin on the hydrogel surface; we examined diffusion through membranes fabricated with and without Sigmacote.

Finally, we measured the diffusion coefficient through our boronic acid glucose sensor PCCA material within the nylon mesh. The PCCA hydrogel within a nylon polymer mesh was hydrolyzed for 3 h, washed thoroughly with a 0.2 M NaCl solution, and attached to 5-amino-2-fluorophenylboronic acid through EDC coupling. After coupling and washing, this hydrogel was used for dialysis measurements. Figure 6 shows the results of a diffusion experiment, displaying the concentration in the receiver cell with time.

For all of these experiments, we find essentially identical glucose concentration differences between the reservoir and receiver cells of ~0.3 mM glucose after 30 min of dialysis, which give essentially identical diffusion coefficients of 3×10^{-6} cm^2/s, close to that in pure water. This clearly indicates that diffusion through the PCCA is similar to bulk water diffusion and that the slow glucose sensing time response is not the result of slow diffusion of glucose into the hydrogel.

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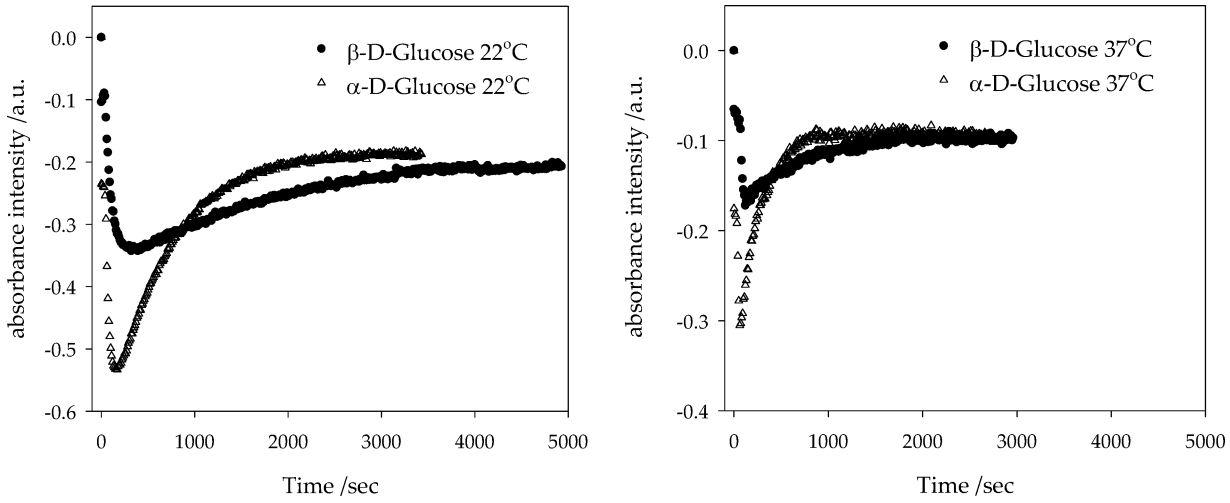
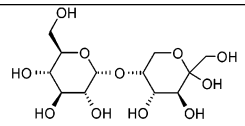
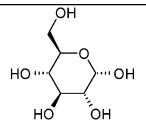


Figure 5. Absorbance changes at 304 nm as function of time after addition of 100 mM freshly prepared α-D-glucose and β-D-glucose to 10 mM 5A-2F-PBA Gly-Gly buffer solution at pH 7.4 with 150 mM NaCl at 22 (left) and 37 °C (right). Path length, 2 mm.

Table 2. Absorbance Saturation Time (at 304 nm) after Mixing 5A-2F-PBA with Glucose

	time (s) to reach initial minimum (±10 s)		time (s) to reach equilibrium (±10 s)	
glucose form	22 °C	37 °C	22 °C	37 °C
β-D-glucose	290	70	3120	1260
α-D-glucose	150	40	1560	540
equilibrated D-glucose	160	40	2520	660

Table 3. Chemical Structure of D-Leucrose and α-D-Glucopyranose

	
D-Leucrose	α-D-glucopyranose

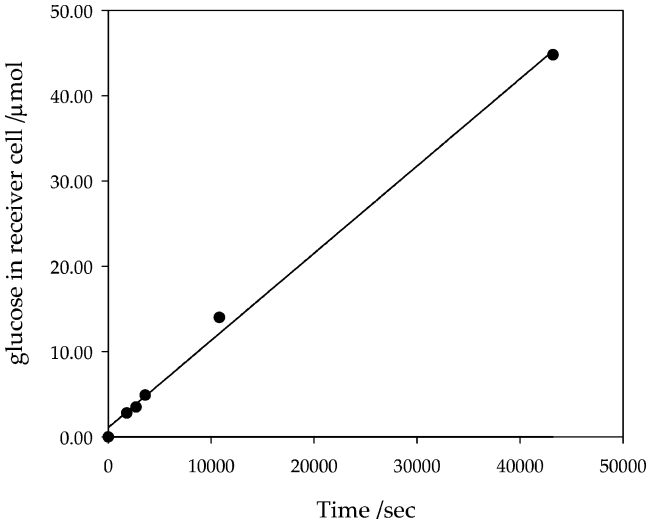


Figure 6. Total measured glucose content of receiver cell as a function of time for diffusion through a hydrogel membrane (slope, 1 μmol/s).

The slowness of the sensor material glucose response could derive from slow bisbidentate binding to the boronate. We tested this possibility by examining the response of the sensor material to compounds that have only one cis-diol (such as sorbitol and fructose), which cannot form bisbidentate cross-links. In this case, binding shifts the equilibrium toward anionic boronates, which cause a diffraction red shift.[4] The red shift in high ionic strength solutions derives mainly from an increased favorability in the free energy of mixing of this ionic hydrogel in water. At low ionic strengths, even larger red shifts occur due to the formation of a Donnan potential.[32] We find that the response of the PCCA to formation of these bidentate complexes is as fast as the response to α-D-glucose and ~5 times faster than to β-D-glucose for both high and low ionic strength solutions. This suggests that the formation of the second attachment to boronates is not a bottleneck.

To probe further whether the slow response time results from a slow step in the formation of the bis bidentate boronate cross-link, we examined the response of the sensor to a reduction in the glucose concentration. The rate of swelling in response to washing glucose out is similar to the rate of binding-induced shrinking. Thus, we conclude that the second binding step is not rate limiting.

We also examined the response rate by monitoring the sensor swelling kinetics in low ionic strength solution, where the repulsion between the boronate anions would prevent formation of the bis-bidentate complex. Thus, only the bidentate glucose−boronate complexes will occur. In this case, the diffraction red shifted at a rate very close to that which occurred during the formation of bisbidentate complex in high ionic strength conditions. This observation eliminates the possibility that the second binding step limits the kinetics. Either the first binding reaction or other hydrogel physicochemical properties are limiting the response kinetics.

Boronate pK_a, Boronate Loading, and Cross-Link Density. We examined the dependence of the sensor response to boronate loading. Either we can either increase the actual phenyl boronic acid concentration in the hydrogel, we can increase the boronate concentration by lowering the pK_a of the phenylboronic acid derivative, or we can raise the solution pH. The affinity of the

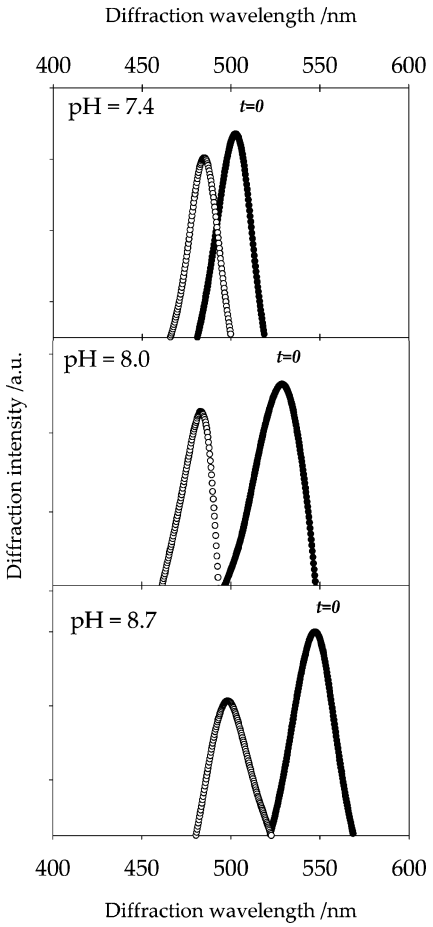


Figure 7. Response of high boronic acid content PCCA glucose sensor to freshly prepared 1 mM β-D-glucose solutions at different pH values: pH 7.4 (upper), pH 8.0 (middle), and pH 8.7 (lower), in a buffered 5 mM Gly-Gly solution with 150 mM NaCl.

tetrahedral anionic boronate form for glucose is much higher than the trigonal boronic acid form.[31]

We optimized the PCCA glucose sensing preparation procedure to increase the boronic acid content by increasing the coupling efficiency and by increasing the hydrogel polymer content to provide more attachment sites for the phenylboronic acid derivative. Figure 7 shows the response of this PCCA glucose sensor to freshly made, nonequilibrated 1 mM β-D-glucose solutions at different pH values. The solutions, which were used 2 min after preparation, did not reach full equilibrium (~3 h) over the measurement period (80 min). It can be seen that the sensor response to 1 mM β-D-glucose increases from a total blue shift of 18 nm at pH 7.4 to a total blue shift of 45 nm at pH 8 and to a total blue shift of 50 nm at pH 8.7 in buffered saline solutions. Little further blue shift occurs for pH values above 8.0. This is consistent with our expected pK_a value of 7.4 for 5A-2F-PBA attached to the PCCA; we earlier measured a pK_a value of 7.8 for the amino derivative of 5A-2F-PBA and expect a 0.4 pH unit decrease[32] of the pK_a upon acetylation of the amine.

Increasing the boronic acid content in the hydrogel also increases the sensor response. By optimizing the coupling

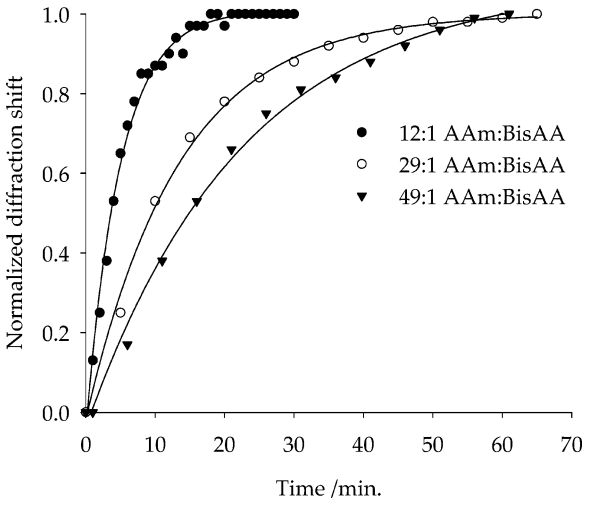


Figure 8. Response kinetics of new PCCA glucose sensors containing different cross-link densities upon exposure to freshly made 1 mM D-glucose in 5 mM Gly-Gly buffer, pH 7.4. 12:1 AAm−BisAA (◆), 29:1 AAm−BisAA (◇), and 49:1 AAm−BisAA (▼). Fit lines are a guide to the eye.

procedure, we increased the boronic acid content in the sensor from ~30 to ~200 mM, which increased the sensitivity to give a 85-nm diffraction shift at pH 7.4 in response to a freshly made 1 mM β-D-glucose solution.

We quantitated the response rate in terms of the time required to achieve 90% of the full response. The normalized response kinetics appear relatively independent of boronate concentration. This was true both when we changed the boronate concentration by changing the loading and also when we changed the boronate concentration by changing the pH.

Cross-link density changes dramatically alter the sensitivity and the normalized response time of the hydrogels. The hydrogel cross-link density determines the hydrogel elasticity, which controls the hydrogel swelling ratio.[9,33] Decreasing the number of cross-links increases the volume change upon swelling. For example, our new PCCA glucose sensing materials prepared with acrylamide to bisacrylamide cross-linker ratios of 49:1, 29:1, and 12:1 (total polymer content of 3.8%) show diffraction blue shifts of 124, 75, and 25 nm, respectively, in response to a freshly made 1 mM D-glucose buffered solution at pH 7.4.

Figure 8 shows the response kinetics of these hydrogel systems. The highest cross-linked system shows a normalized response time 4 times faster than the least cross-linked sensor. It is interesting and disappointing that the absolute response time per nanometer remains fairly constant (2.0−2.7 nm/min). As will be shown later, this result is consistent and supports our finding that the response kinetics is limited by the water and hydrogel network diffusion kinetics.

Hydrophilic−Hydrophobic Balance. We increased the acrylamide, bisacrylamide hydrogel hydrophobicity by incorporating *n*-hexyl acrylate. This study tested the hypothesis that the response rate was limited by the rate of flow of water through the hydrogel and by the collective diffusion time of the hydrogel polymer. This flow rate and polymer diffusion-limited the shrinkage and swelling rates, which were induced by changes in the

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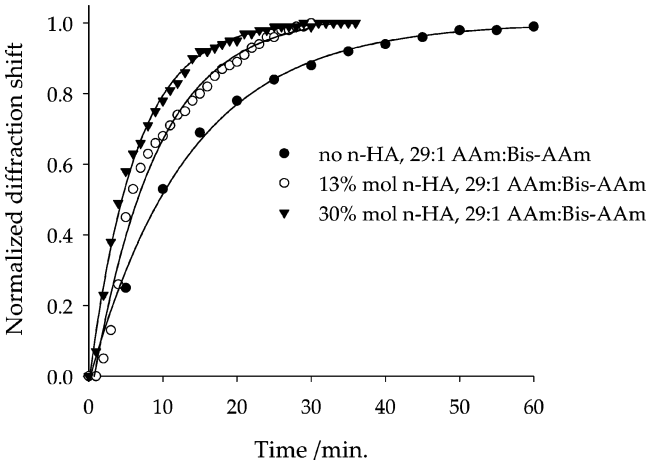

Figure 9. Response kinetics of PCCA glucose sensor containing various amounts of *n*-hexyl acrylate upon exposure to 1 mM D-glucose in 5 mM Gly-Gly buffer, 150 mM NaCl, pH 7.4: (◆) no hexyl acrylate and (○) 13 and (▼) 30 mol % *n*-hexyl acrylate. All hydrogels are composed of 29:1 AAm−BisAA. Fit lines are guide to the eye.

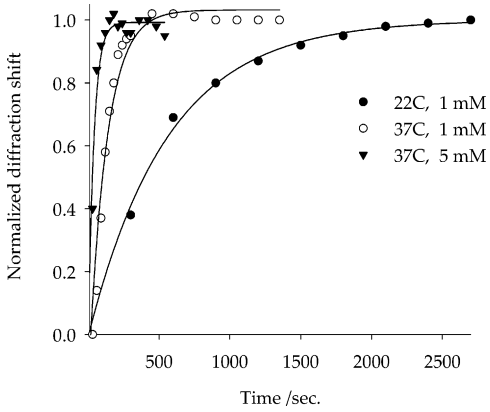

Figure 10. Response kinetics of PCCA glucose sensors containing *n*-hexyl acrylate upon exposure to a freshly prepared D-glucose solution, 5 mM Gly-Gly buffer, 150 mM NaCl, pH 7.4 at various temperatures: (◆) 22 °C, 1 mM; (◇) 37 °C, 1 mM; (▼) 37 °C, 5 mM D-glucose. Fit lines are a guide to the eye.

osmotic pressures induced by changing the number of glucose cross-links. The idea is that a more hydrophobic hydrogel would decrease the friction of water traveling through the polymer, as well as the friction for polymer motion.

Figure 9 shows that incorporation of *n*-hexyl acrylate increases the rate of shrinkage by factors of 2−3, depending on the concentration of *n*-hexyl acrylate. The actual response rate changes significantly from 2 nm/min without hexylacrylate to 3.5 nm/min with hexylacrylate.

Temperature Dependence. The sensor rate of response increases with temperature. Figure 10 shows that the sensor responds 5 times more rapidly at 37 °C than at 22 °C. The response time to a 5 mM glucose concentration (normal blood glucose concentration[34]) decreases to ~1.5 min, which is close to our calculated diffusion-limited time ($t_D = (dx)^2/2D = 26$ s).

We measured the response of these hydrophobic PCCA glucose sensors to 0.2 mM glucose (approximately the normal tear fluid glucose concentration[35]) and found a repeatable rapid

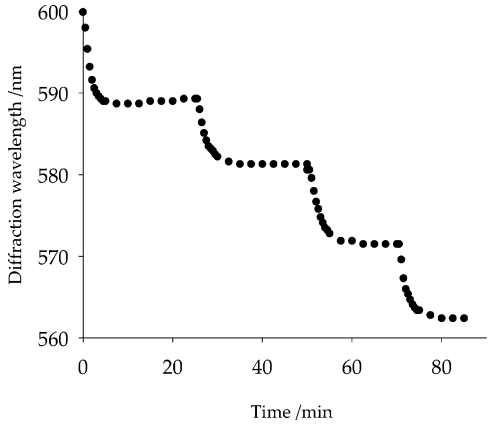

Figure 11. Response kinetics of hexyl acrylate PCCA glucose sensors upon exposure to multiple additions of a freshly prepared 0.2 mM D-glucose solution in 5 mM Gly-Gly buffer, 150 mM NaCl, pH 7.4 at 37 °C. A repeatable rapid blue shift of the diffraction (~10 ± 1 nm) is observed saturating within ~300 s.

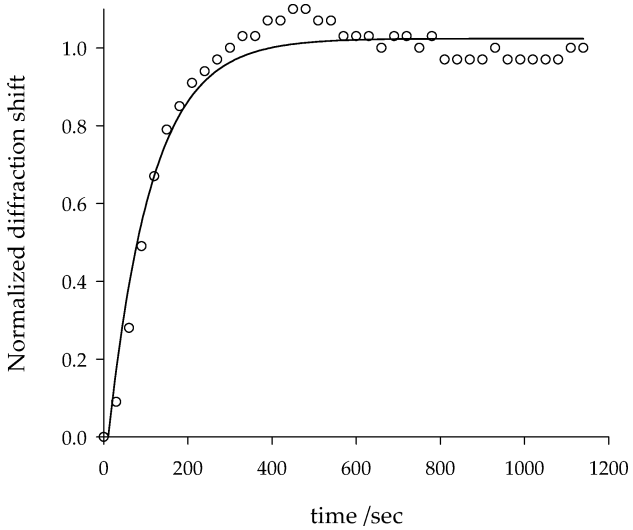

Figure 12. Response kinetics of *n*-hexylacrylate PCCA glucose sensors in artificial tear fluid to a freshly prepared 0.15 mM D-glucose solutions pH 7.4 at 37 °C. A rapid blue shift of the diffraction (~11 nm) is observed saturating within ~300 s.

blue shift of the diffraction (~11 ± 2 nm) saturating within ~300 s. (Figure 11).

From the increase in rate with temperature, we can calculate the apparent activation energy for the hydrogel volume response. For the 5-fold increase in response rate between 22 and 37 °C for D-glucose, we calculate a 20 kcal/mol activation barrier. This activation barrier could derive from single reaction bottleneck slow step in the formation of the glucose boronate complex. It is likely that this activation barrier derives from processes involving collective diffusion times of the hydrogel polymer and that this phenomenological activation barrier is a distributed barrier that derives from a series of bottlenecks. We will continue to examine this issue in the future as we vary the composition of the hydrogel.

Response in Artificial Tear Fluid. We measured the glucose response kinetics of our sensor material in artificial tear fluid solutions in order to determine whether bodily fluids contain species that could confound measurements of glucose.

(34) Burtis, C. A.; Ashwood, E. R. *Tietz Textbook of Clinical Chemistry*, 3rd ed.; W. B. Saunders: Philadelphia, 1999,

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The response kinetics to addition of 0.15 mM glucose in artificial tear fluid[1] at 37 °C is shown in Figure 12. As shown in the previous sections, the temperature significantly accelerates the response kinetics and a rapid blue shift of the diffraction (~11 nm) is observed saturating within ~300 s without any noticeable disruption from the tear fluid matrix.

CONCLUSIONS

We developed a new glucose sensing material that utilizes a bisbidentate glucose−boronate complex, which acts as a hydrogel cross-link. These new sensors do not require sodium chelating agents (such as PEG or crown ethers[1,4]). We demonstrate that it is possible to increase the response kinetics of these glucose sensing materials by graft polymerization of hydrophobic groups onto the hydrogel backbone. We have optimized the response properties so that these sensors can be used in contact lenses to determine glucose in tear fluid. It is shown that glucose mutarotation is an important parameter limiting the sensor response kinetics. We have demonstrated that the sensor is responsive to ~0.15 mM glucose concentrations in artificial tear fluid and that the sensing at body temperatures is significantly faster than at room temperature. These results clearly demonstrate that the *n*-hexylacrylate-modified PCCA glucose sensing material is highly suitable to fabricate a fast, responsive glucose sensor incorporated in contact lenses or ocular inserts.

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