Anal. Chem. **2009,** *81,* 4978–4986

Polymerized Crystalline Colloidal Array Sensing of High Glucose Concentrations

Michelle M. Ward Muscatello, Lee E. Stunja, and Sanford A. Asher*

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

We are developing photonic crystal glucose sensing materials to continuously monitor relatively high glucose concentrations, such as found in blood. We modified our synthetic fabrication methodologies in order to increase the glucose concentration range and to increase the reproducibility of our PCCA fabrication. We have also advanced our understanding of the sensing response by developing a mechanical method to independently determine the hydrogel cross-link density. Our investigation of the sensing mechanism indicates that glucose binding depends mainly on the boronic acid concentrations and affinities. We determined the binding constant of 2-fluoro-5-aminophenyl boronic acid for glucose under physiological conditions. We have examined the dependence of glucose sensing upon interferences by other species that ligand to boronic acids, such as lactate and human serum albumin. We examined the stability of our sensors over a period of weeks at room temperature and demonstrated that we could further stabilize our sensing materials by reversibly dehydrating them for storage.

Hyperglycemia has been shown to be associated with an increased mortality, morbidity, and length of hospital stay for patients suffering from a range of surgical and trauma diagnoses, including acute neurologic disease, cardiac surgery, and stroke.[1−11] In America, diabetes afflicts 23.6 million people (8.0% of the population).[12] People with diabetes have increased hospitalization, with an increased length of stay and a resulting increase in overall care costs.[13−16]

In addition to the need for glucose monitoring in the case of patients with chronic diabetes mellitus, there is a critical need for monitoring glucose levels in nondiabetic acute care patients, since large variations in blood glucose concentration have been observed in nondiabetic patients as a common consequence of surgery or acute illness.[9,17,18] The American Diabetes Association recommends tight control of blood glucose levels for hospitalized critically ill patients.[19] In several recent studies, it has been shown that tight control of blood glucose levels has a significant positive effect on the health of patients in surgical and medical intensive care units, particularly for those suffering from stress-induced hyperglycemia.[20−22] This positive outcome results in substantial cost of care savings.[23]

Establishing a means of reliable, relatively noninvasive, and continuous glucose monitoring would result in markedly improved outcomes for inpatients with diagnosed diabetes mellitus and for those patients who experience acute care induced hyperglycemia.[1,21,22,24] The crucial demand for continuous, accurate, and relatively noninvasive glucose sensing methods has motivated the development of a wide array of sensing materials and methodologies. Many of these approaches to glucose monitoring have utilized boronic acid derivatives to bind glucose.[25−45] For example, boronic acid derivatives have been utilized in the development of

* To whom correspondence should be addressed. E-mail: asher@pitt.edu. Department of Chemistry, University of Pittsburgh, 219 Parkman Avenue, Pittsburgh, PA 15260. Phone: 412-624-8570. Fax: 412-624-0588.

(1) Cakir, M.; Altunbas, H.; Karayalcin, U. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 1402–1402.
(2) Palacio, A.; Smiley, D.; Ceron, M.; Klein, R.; Cho, I. S.; Mejia, R.; Umpierrez, G. E. *J. Hosp. Med.* **2008**, *3*, 212–217.
(3) Capes, S. E.; Hunt, D.; Malmberg, K.; Gerstein, H. C. *Lancet* **2000**, *355*, 773–778.
(4) Smiley, D. D.; Umpierrez, G. E. *South. Med. J.* **2006**, *99*, 580–589.
(5) Conner, T. M.; Flesner-Gurley, K. R.; Barner, J. C. *Ann. Pharmacother.* **2005**, *39*, 492–501.
(6) Estrada, C. A.; Young, J. A.; Nifong, L. W.; Chitwood, W. R. *Ann. Thorac. Surg.* **2003**, *75*, 1392–1399.
(7) Krinsley, J. S. *Mayo Clin. Proc.* **2003**, *78*, 1471–1478.
(8) Garg, R.; Bhutani, H.; Alyea, E.; Pendergrass, M. *Diabetes Care* **2007**, *30*, 993–994.
(9) Matz, K.; Keresztes, K.; Tatschl, C.; Nowotny, M.; Dachenhausenm, A.; Brainin, M.; Tuomilehto, J. *Diabetes Care* **2006**, *29*, 792–797.
(10) Jeremitsky, E.; Omert, L. A.; Dunham, C. M.; Wilberger, J.; Rodriguez, A. *J. Trauma* **2005**, *58*, 47–50.
(11) Ahmann, A. *Endocr. Pract.* **2004**, *10* (2), 53–56.
(12) American Diabetes Association. *Total Prevalence of Diabetes and Pre-Diabetes,* **2008**.
(13) Aro, S.; Kangas, T.; Reunanen, A.; Salinto, M.; Koivisto, V. *Diabetes Care* **1994**, *17*, 1320–1329.
(14) Naslafkih, A.; Sestier, F. *J. Insur. Med.* **2003**, *35*, 102–113.
(15) Ahmann, A. *Endocrinologist* **1998**, *8*, 250–259.
(16) Hirsch, I. B.; Paauw, D. S.; Brunzell, J. *Diabetes Care* **1995**, *18*, 870–878.
(17) McCowen, K. C.; Malhotra, A.; Bistrian, B. R. *Crit. Care Clin.* **2001**, *17*, 107–124.
(18) Mizock, B. A. *Am. J. Med* **1995**, *98*, 75–84.
(19) *Diabetes Care* **2008**, *31*, S5−S11.
(20) Schmeltz, L. R.; DeSanti, A. J.; Thiyagarajan, V.; Schmidt, K.; O'Shea-Mahler, E.; Johnson, D.; Henske, J.; McCarthy, P. M.; Gleason, T. G.; McGee, E. C.; Molitch, M. E. *Diabetes Care* **2007**, *30*, 823–828.
(21) Van den Berghe, G.; Wilmer, A.; Hermans, G.; Meersseman, W.; Wouters, P. J.; Milants, I.; Van Wijngaerden, E.; Bobbaers, H.; Bouillon, R. *N. Engl. J. Med* **2006**, *354*, 449–461.
(22) Krinsley, J. S. *Mayo Clin. Proc.* **2004**, *79*, 992–1000.
(23) Krinsley, J. S.; Jones, R. L. *Chest* **2006**, *129*, 644–650.
(24) Van den Berghe, G. *J. Clin. Invest.* **2004**, *114*, 1187–1195.
(25) Eggert, H.; Frederiksen, J.; Morin, C.; Norrild, J. C. *J. Org. Chem.* **1999**, *64*, 3846–3852.
(26) Lorand, J. P.; Edwards, J. O. *J. Org. Chem.* **1959**, *24*, 769–774.
(27) Fang, H.; Kaur, G.; Wang, B. H. *J. Fluoresc.* **2004**, *14*, 481–489.
(28) James, T. D.; Sandanayake, K.; Shinkai, S. *Supramol. Chem.* **1995**, *6*, 141–157.
(29) James, T. D.; Shinkai, S. *Top. Curr. Chem.* **2002**, *218*, 159–200.
(30) Schneider, H. J.; Kato, K.; Strongin, R. M. *Sensors* **2007**, *7*, 1578–1611.
(31) Wang, W.; Gao, X. M.; Wang, B. H. *Curr. Org. Chem.* **2002**, *6*, 1285–1317.

10.1021/ac900006x CCC: $40.75
Published on Web 05/13/2009

fluorescence, colorimetric, and electrochemical based glucose sensing technologies.[31,36,38]

We recently reported on the development of a boronic acid based glucose sensor,[46−50] which utilizes our previously developed polymerized crystalline colloidal array sensing technology.[51−57] Our photonic crystal hydrogel consists of a mesoscopically periodic array of colloidal particles that self-assembles into a highly ordered crystalline colloidal array (CCA), with a lattice spacing that Bragg diffracts visible light (Figure 1A).[58−65] When the CCA is polymerized within a hydrogel (PCCA), the PCCA optically

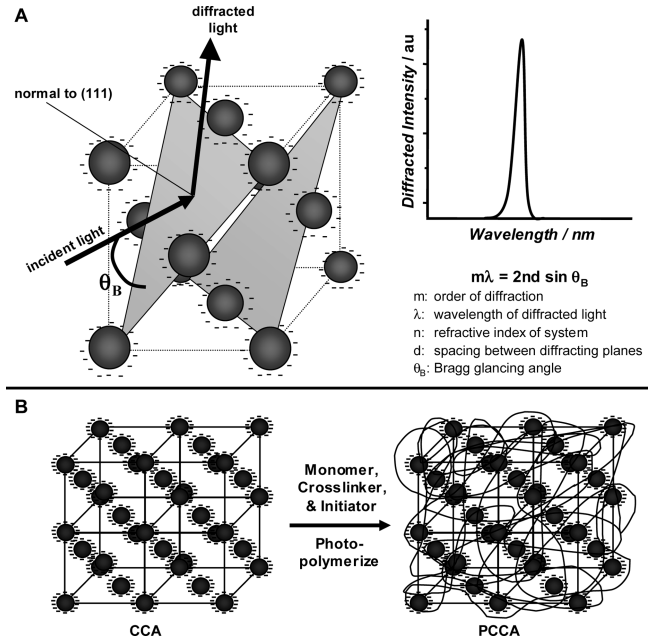

Figure 1. (A) Crystalline colloidal arrays (CCA) assemble due to the electrostatic repulsion between the highly charged, monodisperse polystyrene particles. The spacing between particles is such that they diffract visible light according to Bragg's law. (B) Polymerized crystalline colloidal arrays (PCCA) are formed by polymerizing a hydrogel network around the CCA.

reports on volume changes experienced by the hydrogel, since the observed diffraction wavelength is directly related to the spacing between lattice planes (Figure 1B). We applied this motif to chemical sensing by functionalizing the hydrogel such that changes in the concentration of the analyte of interest actuates changes in the hydrogel volume and, thereby, the diffraction wavelength. We developed intelligent PCCA (IPCCA) for use in the detection of analytes, including glucose,[46−50] cations,[66−69] ammonia,[70] creatinine,[71] pH,[72,73] and organophosphates.[74,75]

For sensing glucose, boronic acid recognition molecules are tethered to the PCCA hydrogel backbone. Additional bis-boronate−glucose cross-links are formed within this originally lightly cross-linked hydrogel upon binding glucose, shrinking the hydrogel volume and blue-shifting the diffraction wavelength in proportion to the concentration of glucose bound (Figure 2).[47] Much of our previous work focused on the development of a glucose-sensing material that functions under physiological pH and ionic strength conditions for the detection of the very low concentrations of glucose, as found in tear fluid

(32) James, T. D. *Creat. Chem. Sensor Syst.* **2007**, *277*, 107–152.
(33) Kondepati, V. R.; Heise, H. M. *Anal. Bioanal. Chem.* **2007**, *388*, 545–563.
(34) Park, S.; Boo, H.; Chung, T. D. *Anal. Chim. Acta* **2006**, *556*, 46–57.
(35) Hovorka, R. *Diabetes Med.* **2006**, *23*, 1–12.
(36) Striegler, S. *Curr. Org. Chem.* **2003**, *7*, 81–102.
(37) James, T. D.; Sandanayake, K.; Shinkai, S. *Angew. Chem.* **1996**, *35*, 1911–1922.
(38) James, T. D. In *Boronic Acids: Preparation and Applications in Organic Synthesis and Medicine*; Hall, D. G., Ed., Wiley-VCH Verlag GmbH: Weinheim, Germany, 2005; pp 441−479.
(39) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *J. Fluoresc.* **2004**, *14*, 617–633.
(40) Shinkai, S.; Takeuchi, M. *Biosens. Bioelectron.* **2004**, *20*, 1250–1259.
(41) Ferrier, R. J. *Adv. Carbohydr. Chem. Biochem.* **1978**, *35*, 31–80.
(42) Springsteen, G.; Wang, B. *Tetrahedron* **2002**, *58*, 5291–5300.
(43) Yang, X. P.; Lee, M. C.; Sartain, F.; Pan, X. H.; Lowe, C. R. *Chem.—Eur. J.* **2006**, *12*, 8491–8497.
(44) Horgan, A. M.; Marshall, A. J.; Kew, S. J.; Dean, K. E. S.; Creasey, C. D.; Kabilan, S. *Biosens. Bioelectron.* **2006**, *21*, 1838–1845.
(45) Yang, X.; Pan, X.; Blyth, J.; Lowe, C. R. *Biosens. Bioelectron.* **2008**, *23*, 899–905.
(46) Alexeev, V. L.; Das, S.; Finegold, D. N.; Asher, S. A. *Clin. Chem.* **2004**, *50*, 2353–2360.
(47) Alexeev, V. L.; Sharma, A. C.; Goponenko, A. V.; Das, S.; Lednev, I. K.; Wilcox, C. S.; Finegold, D. N.; Asher, S. A. *Anal. Chem.* **2003**, *75*, 2316–2323.
(48) Ben-Moshe, M.; Alexeev, V. L.; Asher, S. A. *Anal. Chem.* **2006**, *78*, 5149–5157.
(49) Asher, S. A.; Alexeev, V. L.; Goponenko, A. V.; Sharma, A. C.; Lednev, I. K.; Wilcox, C. S.; Finegold, D. N. *J. Am. Chem. Soc.* **2003**, *125*, 3322–3329.
(50) Asher, S. A.; Alexeev, V. L.; Lednev, I. K.; Sharma, A. C.; Wilcox, C. Intelligent Polymerized Crystalline Colloidal Array Carbohydrate Sensors. U.S. Patent 7,105,352, Sept 12, 2006.
(51) Asher, S. A.; Holtz, J. H. Polymerized Crystalline Colloidal Array Sensor Methods. U.S. Patent 5,854,078, December 29, 1998.
(52) Asher, S. A.; Holtz, J. H. Novel Polymerized Crystalline Colloidal Array Sensors. WO/1998/019787, May 14, 1998.
(53) Asher, S. A.; Holtz, J. H. Novel Polymerized Crystalline Colloidal Array Sensors. WO/1998/041859, Sept 24, 1998.
(54) Holtz, J. H.; Holtz, J. S. W.; Munro, C. H.; Asher, S. A. *Anal. Chem.* **1998**, *70*, 780–791.
(55) Holtz, J. H.; Asher, S. A. *Nature (London, U.K.)* **1997**, *389*, 829–832.
(56) Sunkara, H. B.; Weissman, J. M.; Penn, B. G.; Frazier, D. O.; Asher, S. A. *Polym. Prepr.* **1996**, *37*, 453–454.
(57) Asher, S. A.; Holtz, J.; Liu, L.; Wu, Z. *J. Am. Chem. Soc.* **1994**, *116*, 4997–4998.
(58) Tikhonov, A.; Coalson, R. D.; Asher, S. A. *Phys. Rev. B* **2008**, *77*, 235404/235401–235404/235416.
(59) Asher, S. A.; Weissman, J. M.; Tikhonov, A.; Coalson, R. D.; Kesavamoorthy, R. *Phys. Rev. E* **2004**, *69*, 066619/066611–066619/066614.
(60) Reese, C. E.; Guerrero, C. D.; Weissman, J. M.; Lee, K.; Asher, S. A. *J. Colloid Interface Sci.* **2000**, *232*, 76–80.
(61) Asher, S. A.; Holtz, J.; Weissman, J.; Pan, G. *MRS Bull.* **1998**, *23*, 44–50.
(62) Asher, S. A. Crystalline Colloidal Narrow Band Radiation Filter. U.S. Patent 4,632,517, Dec 30, 1986.
(63) Flaugh, P. L.; O'Donnell, S. E.; Asher, S. A. *Appl. Spectrosc.* **1984**, *38*, 847–850.
(64) Carlson, R. J.; Asher, S. A. *Appl. Spectrosc.* **1984**, *38*, 297–304.
(65) Rundquist, P. A.; Photinos, P.; Jagannathan, S.; Asher, S. A. *J. Chem. Phys.* **1989**, *91*, 4932–4941.
(66) Reese, C. E.; Asher, S. A. *Anal. Chem.* **2003**, *75*, 3915–3918.
(67) Asher, S. A.; Sharma, A. C.; Goponenko, A. V.; Ward, M. M. *Anal. Chem.* **2003**, *75*, 1676–1683.
(68) Asher, S. A.; Peteu, S. F.; Reese, C. E.; Lin, M. X.; Finegold, D. *Anal. Bioanal. Chem.* **2002**, *373*, 632–638.
(69) Baca, J. T.; Finegold, D. N.; Asher, S. A. *Analyst* **2008**, *133*, 385–390.
(70) Kimble, K. W.; Walker, J. P.; Finegold, D. N.; Asher, S. A. *Anal. Bioanal. Chem.* **2006**, *385*, 678–685.
(71) Sharma, A. C.; Jana, T.; Kesavamoorthy, R.; Shi, L.; Virji, M. A.; Finegold, D. N.; Asher, S. A. *J. Am. Chem. Soc.* **2004**, *126*, 2971–2977.
(72) Xu, X.; Goponenko, A. V.; Asher, S. A. *J. Am. Chem. Soc.* **2008**, *130*, 3113–3119.
(73) Lee, K.; Asher, S. A. *J. Am. Chem. Soc.* **2000**, *122*, 9534–9537.
(74) Walker, J. P.; Kimble, K. W.; Asher, S. A. *Anal. Bioanal. Chem.* **2007**, *389*, 2115–2124.
(75) Walker, J. P.; Asher, S. A. *Anal. Chem.* **2005**, *77*, 1596–1600.

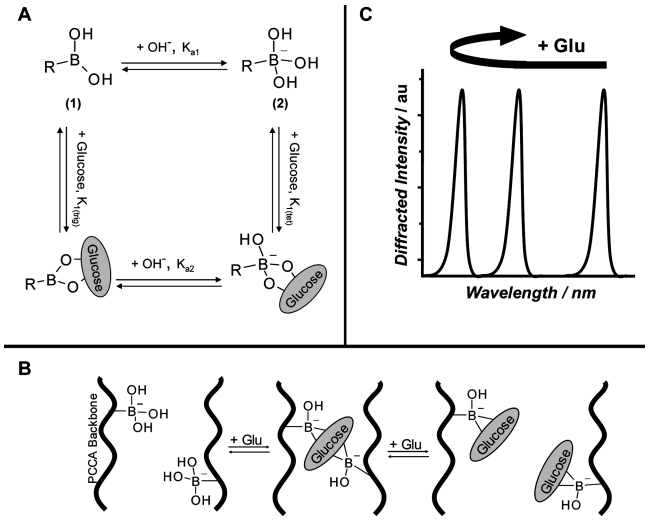

Figure 2. (A) pH-dependent equilibria involved in the 1:1 interaction between boronic acid and glucose. Boronic acid can bind glucose both in its neutral trigonal form (1) and its charged tetrahedral form (2). (B) Boronic acid recognition molecules are tethered to the PCCA hydrogel backbone. Additional cross-links are formed within the hydrogel upon binding glucose, as 2:1 boronate−glucose complexes form. At higher glucose concentrations, these additional cross-links are broken as 1:1 boronate−glucose complexes form. (C) Illustration of how the diffraction from the PCCA reports on the volume changes experienced by the hydrogel. As the hydrogel shrinks due to additional cross-link formation, the diffraction blue-shifts. The bisboronate−glucose cross-links are broken at higher concentrations of glucose. Thus, the hydrogel swells and the diffraction then red-shifts.

(~0.1 mM).[46,76] In the work here, we further developed our sensing material for monitoring higher glucose concentrations, such as found in blood (~5 mM).[77]

EXPERIMENTAL SECTION

Materials. 2,2-Diethoxyacetophenone (98%, DEAP) was purchased from Acros Organics. Phosphate buffered saline (0.1 M phosphate, 0.15 M NaCl, pH 7.2, PBS), MES buffered saline [0.1 M 2-(morpholino)ethanesulfonic acid, 0.9% NaCl, pH 4.7, MES], and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Pierce Biotechnology. Acrylamide (98%, AA), *N*,*N*′-methylenebisacrylamide (98%, BisAA), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (98%, TEMED), sodium L-lactate (99%), D(+)-glucose (99.5%), and Alizarin Red S (ARS) were purchased from Sigma-Aldrich. HCl, NaOH, and NaCl were purchased from J.T. Baker. Dimethyl sulfoxide (DMSO) was purchased from Thermo Fisher Scientific, Inc. 5-Amino-2-fluorophenylboronic acid (98%, BA) was purchased from Asymchem. Gel support films were purchased from Bio-Rad Laboratories. All chemicals were used as received. Deionized water (Barnstead Nanopure Water Purification System) was used for solution preparation.

CCA Preparation. Monodisperse, highly charged polystyrene colloids were prepared via a modified emulsion polymerization as reported elsewhere,[60] with additional initiator and charged monomer being added near the end of the polymerization process to increase the surface charge on the particles. We used ~16% w/w suspensions of 145 nm diameter CCA dispersed in pure water. The colloidal particles were cleaned via dialysis against deionized water and subsequently shaken with ion-exchange resin.

PCCA Hydrogel Preparation. The PCCA was synthesized by free radical polymerization in the absence of oxygen. In a typical recipe, 0.1 g of AA, 0.002 g of BisAA, ion-exchange resin, and 2.0 g of CCA were vortexed together in a small glass vial. The vial was wrapped in Al foil, and 20 μL of DEAP (20% in DMSO) was added. The solution was vortexed again. The polymerization solution and polymerization cell, which consisted of two quartz plates separated by a 125 μm parafilm spacer with a gel support film containing surface vinyl groups used as one face of the cell, were degassed in a vacuum dissector and then filled with nitrogen in a glovebag.

Within the glovebag, the polymerization solution was centrifuged, injected into the cell, and then sealed within a Ziploc bag, so as to exclude oxygen during polymerization. The system was photopolymerized with UV light from two Blak Ray (365 nm) mercury lamps. The resulting PCCA, which was covalently affixed to the gel support film during polymerization, was rinsed with large quantities of pure water.

For the determination of permanent effective cross-link density, the PCCA were prepared using a 250 μm parafilm spacer and without the gel support film.

PCCA Functionalization. Amides on the PCCA hydrogel backbone were converted to carboxylates via hydrolysis in a 0.1 M NaOH and 10% v/v TEMED solution for 1.5 h, unless otherwise noted. The hydrolyzed PCCA was washed extensively with 150 mM NaCl solution. A NaCl solution was used to avoid stress on the immobilized hydrogel due to hydrogel swelling which would occur in pure water due to the Donnan potential resulting from the immobilized carboxylate groups on the hydrogel backbone.

In order to attach BA recognition groups to the hydrogel backbone, the PCCA was immersed in a MES buffer solution containing 25 mM BA and 25 mM EDC (pH 4.7) for 1 h. Additional EDC was then added to the initial coupling solution, to bring the total concentration of EDC to 50 mM, so as to ensure complete coupling between the hydrogel carboxyl groups and the BA amino groups. The IPCCA were extensively washed with a PBS buffer solution (pH 7.4).

Diffraction Measurements. Diffraction measurements were conducted at a fixed 90° glancing angle utilizing an Ocean Optics USB2000-UV−vis spectrometer, a LS-1 tungsten halogen light source, and an R-series fiber optic reflection probe. Experiments were conducted in a covered Petri dish, so as to avoid evaporation. Holes were drilled in the lid and secured with parafilm, allowing access for the reflection probe and addition/removal of solutions.

To characterize the response of the IPCCA to changes in glucose concentration, the hydrogel was first equilibrated in PBS buffer (adjusted to pH 7.4), unless otherwise noted. Glucose was added to the testing solution by removing a small amount of buffer from the Petri dish, dissolving the glucose within that buffer, and then returning this solution to the Petri dish. After addition of glucose, the diffraction was monitored until it stabilized. The IPCCA was rinsed between sensing runs with copious amounts of PBS buffer. The diffraction response shown is the average response at each glucose concentration for three separate pieces of a single fabricated IPCCA sensor. Although each measurement

(76) Baca, J. T.; Finegold, D. N.; Asher, S. A. *Ocul. Surf.* **2007**, *5*, 280–293.
(77) Burtis, C. A., Ashwood, E. R., Eds. *Tietz Textbook of Clinical Chemistry*, 3rd ed.; W. B. Saunders: Philadelphia, PA, 1999.

of the response of the sensor to a different solution condition, such as the presence of lactate for example, utilized sensor pieces originating from the same hydrogel, the different conditions examined were tested on different hydrogel samples.

To ensure that a lack of a mutarotation equilibrium of the freshly prepared glucose solutions did not bias the diffraction response of the sensor,[48] we compared the response to the solution prepared as discussed above to that which occurred upon addition of a pre-equilibrated concentrated glucose solution, prepared in PBS buffer (pH 7.4) several hours prior to the addition. Although the kinetics of the response differed, the final diffraction shift was essentially identical. Thus, all sensing runs reported here were accomplished by the dissolution of the solid within the equilibration buffer at the time of testing.

PCCA Permanent Effective Cross-Link Determination. Oscillatory shear measurements were performed with an AR2000 rheometer (TA Instruments) using a 25 mm parallel plate geometry, with the temperature being maintained at 25 °C by a Peltier stage. After loading the sample, water was added around the edge of the sample to prevent evaporation of the water in the hydrogel during the experiment. The storage modulus was measured using a frequency-sweep between 0.1 to 100 rad/s at a fixed oscillatory shear strain of 5%. As the material was analyzed at low strains, no further modification of the stage was necessary to prevent slip of the material.

BA Binding Constant Determination. The binding constant of BA to glucose was determined using an ARS competitive binding assay method.[42,78,79] The binding constant of BA to ARS was first determined by titrating an ARS solution (9×10^{-6} M ARS) with a BA solution (2×10^{-3} M BA, 9×10^{-6} M ARS) and monitoring the absorbance changes upon complex formation. The BA−ARS complex solution (2×10^{-3} M BA, 9×10^{-6} M ARS) was then titrated with a BA−ARS−glucose solution (2×10^{-3} M BA, 9×10^{-6} M ARS, 1.2 M glucose) to determine the binding constant of BA to glucose. All studies were carried out in PBS at pH 7.4. The reported values are the average of duplicate titrations.

RESULTS AND DISCUSSION

Sensing Response Dependence on Boronic Acid Concentration. We investigated the dependence of the glucose sensing response on the concentration of BA incorporated in the IPCCA. We previously found that sensors with low concentrations of incorporated boronic acid recognition groups utilized a supramolecular bisbidentate glucose−boronate complex, where Na^+ chelating agents [polyethylene glycol (PEG) or crown ethers] were required for cross-link formation.[46,47] We subsequently found that glucose−boronate bisbidentate cross-links occurred in the absence of such chelating agents for high concentrations of boronic acid.[48]

In our new studies reported here, we surprisingly find that Na^+ chelating agents are unnecessary for a response to glucose in high ionic strength solutions, even for low concentrations of incorporated BA groups. As seen in Figure 3A, sensors with both relatively low and high BA concentrations exhibit a blue-shift in the diffracted wavelength upon glucose binding in the absence of PEG or crown ethers. IPCCA with different concentrations of BA were prepared by hydrolyzing the material for different times (0.5 and 1.5 h) prior to coupling with BA. As the boronic acid is tethered to the hydrogel through the carboxyl groups produced during hydrolysis, this resulted in sensors with different concentrations of incorporated BA.

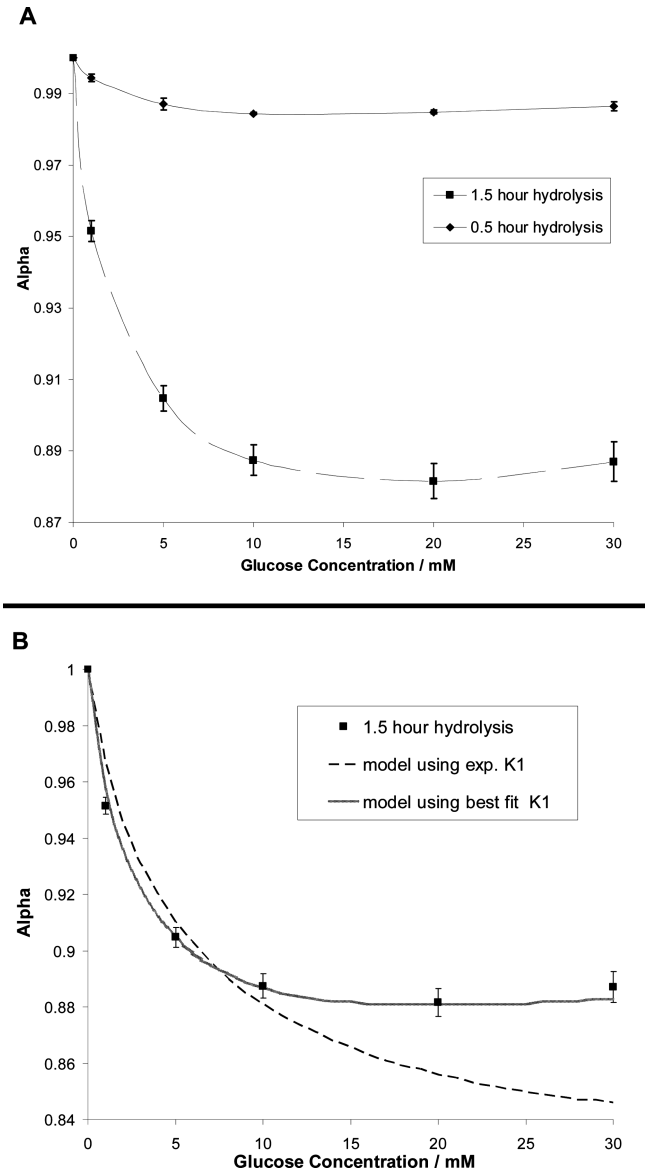

Figure 3. (A) The magnitude of the diffraction shift depends on the concentration of boronic acid (BA) attached to the hydrogel backbone. The mechanism of the response is the same, both at low (0.5 h hydrolysis) and higher (1.5 h hydrolysis) BA concentrations. The additional cross-links formed within the hydrogel as the tethered BA groups bind glucose in 2:1 complexes shrinks the hydrogel volume, blue-shifting the diffraction. (Lines added to aid the eye.) (B) With the use of the experimentally determined value of the association constant of BA for glucose (dashed line), our model somewhat fits the experimental data for lower glucose concentrations but fails at higher glucose concentrations. We, however, find that we can well fit the experimental data if we use larger association constants (solid line), which indicates that the effective association constants of the bound complexes are affected by the attached hydrogel and that solution equilibria are too simplistic to successfully model the interactions within the hydrogel matrix.

(78) Springsteen, G.; Wang, B. *Chem. Commun.* **2001**, 1608–1609.
(79) Dowlut, M.; Hall, D. G. *J. Am. Chem. Soc.* **2006**, *128*, 4226–4227.

We characterized the response of these materials by plotting the linear deformation factor, $\alpha = \lambda/\lambda^*$, where λ and λ^* are the diffracting wavelengths at the glucose concentration of interest and in the absence of glucose, respectively. The observed diffraction wavelength decrease is due to the additional cross-links formed within the hydrogel as the tethered boronic acid moieties bind glucose in 2:1 complexes. The increase in the effective cross-link concentration shrinks the hydrogel volume, blue-shifting the diffraction.

The most likely explanation of our earlier erroneous conclusions is that the sensors previously prepared in the absence of chelators all contained an insufficient concentration of boronic acid, which biased our results. In fact, our earlier studies did show occasional unexplained, but rare, failures to couple boronic acid derivatives to the hydrogel.

Revised Modeling of IPCCA Sensing Response. The sensing mechanism employed in our IPCCA material, which in this case employs bisbidentate complex formation, can be modeled[47,71,72,80,] by utilizing Flory's model[82] for the swelling of polymer network structures by requiring that the total osmotic pressure at equilibrium equal to zero:

$$\Pi_T = \Pi_M + \Pi_E + \Pi_{Ion} = 0 \quad (1)$$

Π_M is the osmotic pressure arising from the change in free energy of mixing:

$$\Pi_M = -\frac{\partial \Delta G_M}{\partial V} = -\frac{RT}{V_s}\left[\ln\left(1 - \frac{V_0}{V}\right) + \frac{V_0}{V} + \chi\left(\frac{V_0}{V}\right)^2\right] \quad (2)$$

Π_E is the osmotic pressure arising from the change in the elastic free energy:

$$\Pi_E = -\frac{\partial \Delta G_E}{\partial V} = -RTv_e\left[\left(\frac{V_m}{V}\right)^{1/3} - \frac{1}{2}\frac{V_m}{V}\right] - RTc_{B_2G} \quad (3)$$

and Π_{Ion} is the osmotic pressure due to the Donnan potential, arising from the difference in mobile ion concentration inside and outside of the hydrogel:

$$\Pi_{Ion} = RT(c_+ + c_- - c_+^* - c_-^*) \quad (4)$$

where R is the universal gas constant, T is the temperature, χ is the Flory–Huggins interaction parameter, V_s is the molar volume of the solvent, v_e is the effective cross-link density of the hydrogel network, c_{B_2G} is the concentration of the additional cross-links formed upon glucose binding, V is the current volume of the hydrogel, V_m is the volume of the relaxed hydrogel network, V_0 is the volume of the dry hydrogel network, c_+ and c_- are the concentrations of mobile cations and anions inside the hydrogel, and c_+^* and c_-^* are the concentrations of mobile cations and anions outside the hydrogel.

As the Donnan potential is negligible in high ionic strength solutions and should insignificantly affect the hydrogel volume, the total osmotic pressure thereby results from changes in the free energies of mixing and elasticity. At equilibrium, $\Pi_M + \Pi_E = 0$ In addition, we ignore changes in the value of χ upon glucose binding, and we assume that the volume changes experienced by the hydrogel derive primarily from changes in the effective number of cross-links, which are formed and broken upon changes in the glucose concentration.[47]

The equilibria involved in glucose binding by boronic acid are complex, as glucose can be bound by both the neutral trigonal and anionic tetrahedral forms of boronic acid. For simplicity's sake, at constant pH, we assume two equilibria and two effective binding constants to denote the overall binding of glucose by boronic acid compounds (in both trigonal and tetrahedral forms) in 1:1 and 2:1 complexes:

$$B + G \overset{K_1}{\longleftrightarrow} BG \quad K_1 = \frac{[BG]}{[B][G]} \quad (5)$$

$$2B + G \overset{K_2}{\longleftrightarrow} B_2G \quad K_2 = \frac{[B_2G]}{[B]^2[G]} \quad (6)$$

Using the total concentration of boronic acid sites within the IPCCA

$$C_T = [B] + [BG] + 2[B_2G] = [B] + K_1[B][G] + 2K_2[B]^2[G] \quad (7)$$

we determine the concentration of glucose–boronate cross-links within the IPCCA, $[B_2G]$,

$$c_{B_2G} = [B_2G] = K_2[B]^2[G] \quad (8)$$

at any given glucose concentration, [G].

The volume changes experienced by the IPCCA upon glucose binding are modeled by calculating the concentration of 2:1 boronate–glucose complexes formed as a function of the glucose concentration and making use of the relationship between hydrogel volume and diffraction wavelength:

$$\left(\frac{V}{V^*}\right)^{\frac{1}{3}} = \frac{\lambda}{\lambda^*} \quad (9)$$

where V and V^* are the hydrogel volumes at the glucose concentration of interest and in the absence of glucose, respectively.

We are now able to constrain our model because we directly measured the effective cross-link density of the hydrogel in the absence of glucose. We found an effective permanent cross-link concentration of 0.8 mM, which is ~2-fold smaller than the 1.46 mM previously estimated from less reliable elastic deformation measurements.[73]

We experimentally determined the permanent effective cross-link density by using mechanical measurements to determine the shear storage modulus (G'). The effective cross-link density, v_e,

(80) Yan, F.; Asher, S. *Anal. Bioanal. Chem.* **2007**, *387*, 2121–2130.
(81) Goponenko, A. V.; Asher, S. A. *J. Am. Chem. Soc.* **2005**, *127*, 10753–10759.
(82) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953.

can be determined from the experimentally determined shear storage modulus and the polymer volume fraction (ϑ):[82,83]

$$G' = v_e RT\vartheta^{\frac{1}{3}} \quad (10)$$

We estimated the Flory−Huggins interaction parameter, $\chi = 0.513$, from hydrogel swelling in the absence of glucose. The concentration of BA within the hydrogel was determined by boron elemental analysis (Columbia Analytical Services, Tucson, AZ).

We determined the association constant of 5-amino-2-fluorophenylboronic acid and glucose by using an ARS competitive assay.[42,78,79,84] The effective binding constant of BA to glucose in PBS at pH 7.4 was found to be $K_1 = 16\ M^{-1}$, which is much lower than the binding constant of 370 M^{-1} we used to model the response of our previous generation of photonic crystal glucose sensors that utilized 4-amino-3-fluorophenylboronic acid.[46,47] However, this $K_1 = 16\ M^{-1}$ value is close to those of other phenylboronic acid derivatives recently determined.[42,79]

We attempted to model our sensing response using this $K_1 = 16\ M^{-1}$ association constant for 1:1 boronate−glucose complex formation and a slightly smaller value of $K_2 = 12\ M^{-2}$ for 2:1 boronate−glucose complex formation. We utilized a lower K_2 value to account for the expected decreased binding constant to the second glucose cis diol groups and to account for an effective decrease in the availability of the second boronic acid derivative due to the constraints imposed by the hydrogel. Figure 3B illustrates our best modeling of the IPCCA diffraction response for glucose, for an incorporated BA concentration of 170 mM and these K_1 and K_2 values. Our model somewhat fits the experimental data for lower glucose concentrations but fails at higher glucose concentrations. We, however, find that we can well fit the experimental data if we use a $K_1 = 50\ M^{-1}$ and $K_2 = 18\ M^{-2}$, which indicates that the effective association constants of the bound complexes are affected by the attached hydrogel. It is no surprise that K_2 would be decreased compared to K_1, as discussed above. The increase in K_1 is more difficult to explain; it could result from a stabilization of the complex by the hydrogel environment.

Our previous modeling required higher values of K_2 to model the large diffraction blue-shifts observed at that time for low glucose concentrations. We now find that the measurement time frame was insufficient; our sensors had not reached equilibrium. We find that the hydrogel diffraction does not plateau at the point of the furthest blue-shift; rather, the hydrogel then slowly red-shifts back before finally plateauing (Figure 4A).

This prompt large blue-shift followed by a slow red-shift most likely results from hydrogel polymer relaxation. This is confirmed by the fact that the kinetics are sped up by exercising the hydrogel (Figure 4B). We previously demonstrated that we can further speed up the kinetics by modifying the hydrogel composition and working at physiological temperatures.[48]

Initially the hydrogel volume is dominated by glucose−bisboronate linkages involving instantaneous nearest neighbor cross-links formed at the point of glucose binding. At longer times, the glucose binding hydrogel complexation equilibrates such that the equilibrium cross-linking network stores the minimum elastic energy. Since the observed diffraction monitors the lattice spacing within the first ~50 layers of the embedded CCA, we are monitoring the hydrogel polymer relaxation within an outermost layer of the sensor. This relaxation process is obviously slower than the initial formation of glucose cross-links.

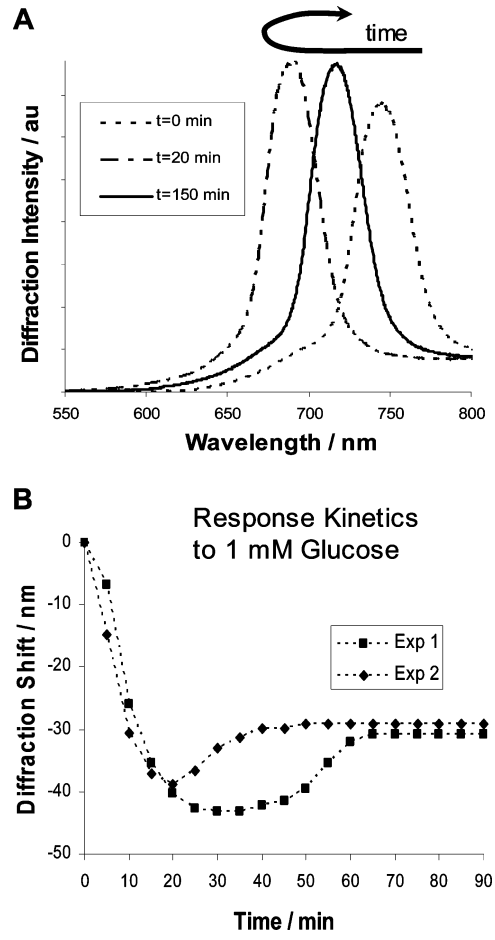

Figure 4. (A) The diffraction of the IPCCA is shown at 0, 20, and 150 min after addition of 1 mM glucose. Initially, the sensor diffraction blue-shifts in response to the addition of 1 mM glucose. Later, the diffraction begins to slowly red-shift before finally stabilizing. The prompt large blue-shift followed by a slow red-shift likely results from polymer relaxation. Initially the hydrogel volume is dominated by glucose−bisboronate linkages involving nearest neighbor cross-links formed initially at the moment of glucose exposure. At a longer time scale, the glucose binding equilibrates within the system, such that the equilibrium cross-links store the minimum elastic energy, which results in an increased equilibrium gel volume near the surface compared to that initially formed, which red-shifts the diffraction. (B) When a sensor is put through a second sensing run (after rinsing in buffer), the initial blue-shift is smaller and equilibrium is reached in a shorter time frame. (Lines are added to aid the eye.)

Sensing Response Dependence on L-Lactate. Our glucose sensor must be free from significant interferences from the nonglucose constituents of blood. Lactate, a known constituent of blood, is known to bind to boronic acid derivatives.[85−87] We measured the impact of a range of lactate concentrations (2−45 mM) on our IPCCA glucose sensing response (Figure 5A). These results clearly show that competitive binding of lactate to boronic acids within our IPCCA reduces the bisbidentate glucose cross-link concentrations. The decrease in response to glucose and deviation from saturation at high

(83) Erman, B.; Mark, J. E. *Structures and Properties of Rubberlike Networks*; Oxford University Press: New York, 1997.
(84) Connors, K. A. *Binding Constants: The Measurements of Molecular Complex Stability*; John Wiley & Sons, Inc.: New York, 1987.

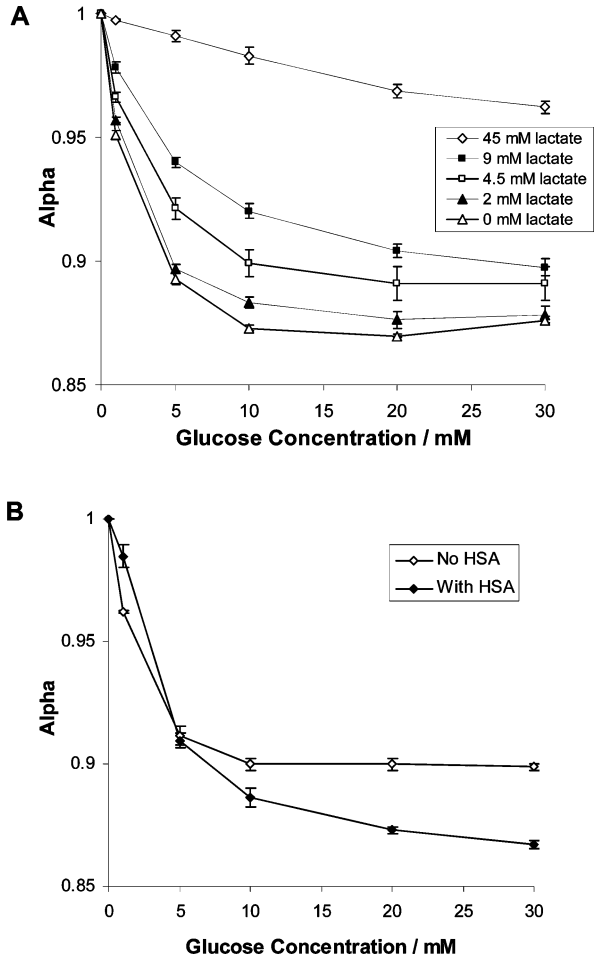

Figure 5. (A) The average experimentally determined diffraction response of a glucose-sensitive IPCCA at varying concentrations of glucose and L-lactate. The alteration in the IPCCA sensing response to glucose, due to the competitive binding of the boronic acid to lactate, becomes more pronounced at higher lactate concentrations. (Lines are added to aid the eye. The 0 mM lactate data points are the average of four sensing runs where the sensors were taken from two separate pieces of an IPCCA fabricated in the same fashion as the IPCCA used for the sensing runs in the presence of lactate.) (B) IPCCA response dependence on human serum albumin (HSA). At concentrations of HSA comparable to the average HSA concentration in serum, the overall sensitivity is increased due to changes in the free energy of mixing of the system, while the lack of a plateau in response to the range of glucose concentrations tested is due to competitive binding between the boronate and glycosylated protein in solution. (Lines are added to aid the eye.)

glucose concentrations becomes more pronounced at higher lactate concentrations.

This competitive binding contribution can be modeled by adding the additional equilibrium that involves boronate−lactate binding:

$$B + L \overset{K_3}{\leftrightarrow} BL \quad K_3 = \frac{[BL]}{[B][L]} \tag{11}$$

Now the total concentration of boronic acid sites within the IPCCA is

$$C_T = [B] + [BG] + 2[B_2G] + [BL] = [B] + K_1[B][G] + 2K_2[B]^2[G] + K_3[B][L] \tag{12}$$

such that the concentration of additional cross-links formed will be decreased by the formation of lactate−boronate complexes.

Since the lactate concentration in a resting adult lies between 0.36−0.75 mM,[77] our Figure 5A data indicate that lactate will insignificantly impact IPCCA monitoring of glucose in blood, as there was only a 0.5% decrease in α for 5 mM glucose in the presence of 2 mM lactate. In addition, our IPCCA could utilize boronic acid derivatives that selectively bind glucose over lactate, such as the 2-acrylamidophenylboronate (2APB) derivative synthesized by Lowe et al.[43] They utilized a phenylboronate derivative stabilized in the tetrahedral form through interaction with a neighboring ortho group. Since boronic acids preferentially bind glucose in the tetrahedral form and lactate in the trigonal form, interference from lactate is reduced.[86,88,89] The stabilization of this boronic acid derivative in the tetrahedral form has the added benefit of alleviating any pH dependence of glucose binding.

Interestingly, the shrinkage of the sensor in response to glucose does not saturate at the higher lactate concentrations. This indicates that a competitive binding based sensing mechanism could be used to fabricate an IPCCA capable of monotonically responding to glucose over a large concentration range. By attachment of a competitor species within the hydrogel material and optimizing its concentration and binding constant, a near linear response of the sensor to glucose concentrations could be achieved.

Sensing Response Dependence on Human Serum Albumin. Human serum albumin (HSA), the most abundant protein in human serum,[77] is known to undergo glycosylation upon exposure to glucose via reaction between the sugar aldehyde group and the free amino groups of the protein.[90−92] We characterized the dependence of our IPCCA glucose response on the presence of HSA at the concentration typically found in serum (44 g/L).[77] Figure 5B shows that HSA addition to the PBS solution increases the sensitivity at higher glucose concentrations, increasing the IPCCA response spectral window. The diffraction response, surprisingly, does not plateau at the higher glucose concentrations as it does in the absence of HSA.

The decreased response to addition of 1 mM glucose presumably results from "consumption" of the glucose by HSA glycosylation.[93−95] This lowers the concentration of free glucose in solution.

The overall increase in the spectral window is related to changes in the free energy of the IPCCA upon HSA binding. The boronic

(85) Gray, C. W.; Houston, T. A. *J. Org. Chem.* **2002**, *67*, 5426–5428.
(86) Sartain, F. K.; Yang, X. P.; Lowe, C. R. *Anal. Chem.* **2006**, *78*, 5664–5670.
(87) Wiskur, S. L.; Lavigne, J. L.; Metzger, A.; Tobey, S. L.; Lynch, V.; Anslyn, E. V. *Chem.—Eur. J.* **2004**, *10*, 3792–3804.
(88) Babcock, L.; Pizer, R. *Inorg. Chem.* **1980**, *19*, 56–61.
(89) Friedman, S.; Pace, B.; Pizer, R. *J. Am. Chem. Soc.* **1974**, *96*, 5381–5384.
(90) Neglia, C. I.; Cohen, H. J.; Garber, A. R.; Ellis, P. D.; Thorpe, S. R.; Baynes, J. W. *J. Biol. Chem.* **1983**, *258*, 4279–4283.
(91) Day, J. F.; Thorpe, S. R.; Baynes, J. W. *J. Biol. Chem.* **1979**, *254*, 595–597.
(92) Olufemi, S.; Talwar, D.; Robb, D. A. *Clin. Chim. Acta* **1987**, *163*, 125–136.
(93) Yatscoff, R. W.; Tevaarwerk, G. J. M.; Macdonald, J. C. *Clin. Chem.* **1984**, *30*, 446–449.
(94) Guthrow, C. E.; Morris, M. A.; Day, J. F.; Thorpe, S. R.; Baynes, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4258–4261.
(95) Mohamadi-Nejad, A.; Moosavi-Movahedi, A. A.; Hakimelahi, G. H.; Sheibani, N. *Int. J. Biochem. Cell Biol.* **2002**, *34*, 1115–1124.

acid moieties on the hydrogel are capable of binding HSA, even in its nonglycosylated state.[96−98] When the IPCCA is transferred from PBS to a PBS solution containing HSA, the wavelength of diffraction red-shifts. The complexing of the tethered boronic acid to the soluble protein increases the free energy of mixing induced osmotic pressure, swelling the hydrogel. With the use of the diffraction values for the hydrogel swelling in the absence of glucose, we estimate a value of $\chi = 0.505$ for the system containing HSA. We modeled the response of our sensor using this new χ value, which predicts an increased glucose response consistent with that experimentally observed. The osmotic compressibility of hydrogel networks is known to depend strongly on χ, where decreases in χ result in increased compressibility.[83]

The nonplateau in diffraction response can be attributed to competitive binding. As the albumin is a relatively large molecule compared to glucose, the initial association within the hydrogel hinders the accessibility of boronic acid sites that would normally be accessible to glucose. The addition of glucose, and subsequent glycosylation of the protein, elevates its role as a competitive binder. This is similar to the competitive binding phenomenon observed for lactate, as described above.

It is clear from Figure 5B that the presence of HSA changes the diffraction response of the IPCCA to free glucose. As the concentration of glycosylated protein in circulation can vary, especially for those experiencing hyperglycemia,[93−95] these proteins should be excluded from the sensor by a dialysis membrane for practical *in vivo* sensing. A dialysis membrane can be straightforwardly included in an optrode constructed similarly to that previously reported by our group.[54] IPCCA diffraction would be monitored from the side opposite of that in contact with the blood, alleviating any effect of the membrane or absorption from blood on the diffraction measurement.

Sensing Response Dependence on Ionic Strength. We examined the ionic strength dependence of the PCCA glucose sensor response in PBS solutions at total ionic strengths (TIS) of 60, 300, and 450 mM. Figure 6A shows that decreasing the TIS below that of the PBS solution (450 mM) results in a decreased diffraction blue-shift.

The decreased blue-shift at low TIS results from the competition between the hydrogel shrinking, due to the additional cross-link formation, competing with the hydrogel swelling, due to the formation of the Donnan potential caused by the immobilization of a relatively high concentration of boronate anions on the hydrogel backbone, as boronic acid predominantly binds glucose in its anionic boronate form.

The Donnan potential arises from the concentration difference between the mobile counterions inside and outside of the hydrogel. When, as in this case, there is a high concentration of immobilized charged groups and their counterions within the hydrogel, the Donnan-induced osmotic pressure can be large even at relatively high ionic strengths. IPCCA response dependence on the ionic strength can be taken into account by including the Donnan potential osmotic pressure within our model:[72,73]

$$\Pi_{\mathrm{Ion}} = RT[iC_{\mathrm{BA}} - 2(C_{\mathrm{S}}{}^{*} - C_{\mathrm{S}})] \qquad (13)$$

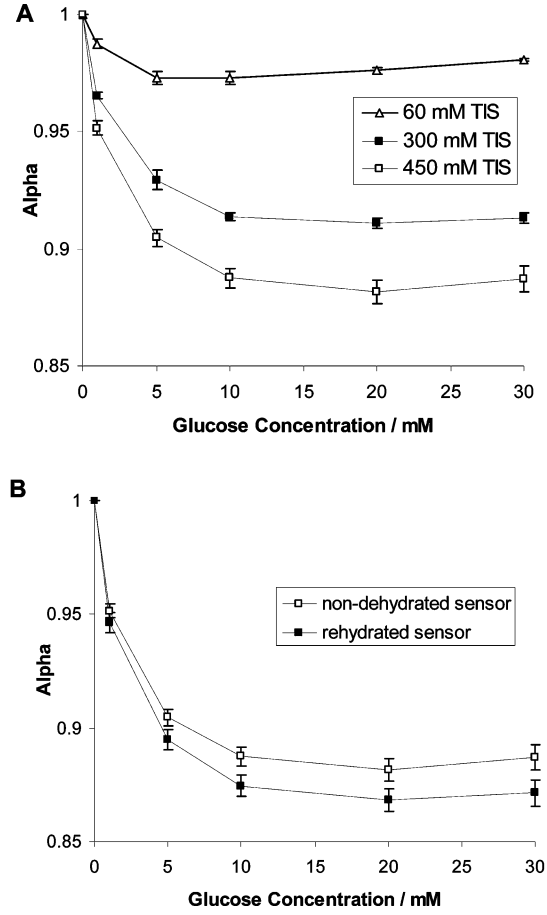

Figure 6. (A) Dependence of IPCCA response on total ionic strength at pH 7.4. At low ionic strength, the swelling due to the Donnan potential is large, thereby resulting in a decrease in the overall shrinking and blue-shifting in response to the increase in cross-link density upon glucose binding. (Lines added to aid the eye.) (B) IPCCA response dependence on rehydration. The IPCCA retains sensing capability after drying in the presence of a concentrated glucose solution. The weight percent of glucose is sufficient to prevent the irreversible collapse of the acrylamide hydrogel network. The increase in spectral response after rehydration is due to the liberation of previously inaccessible BA sites during the "conditioning step" of equilibration in 45 wt % glucose. (Lines are added to aid the eye.)

where i is the degree of boronic acid ionization times its charge, C_{BA} is the concentration of boronic acid groups within the IPCCA, $C_{\mathrm{S}}{}^{*}$ is the concentration of mobile ions within the IPCCA, and C_{S} is the concentration of mobile ions in the bulk solution. We will include the dependence on this Donnan potential in future modeling of this system.

The major electrolytes found in extracellular fluids are Na^+ and Cl^-, which together comprise the greatest fraction of osmotically active constituents of plasma. The reference intervals for Na^+ and Cl^- are 136−145 mM and 98−107 mM, respectively.[77] Taking only these intervals into consideration, one can expect a ±4% variation in the total ionic strength of plasma. As the dependence of α on TIS is linear over the concentrations studied, such a variation in TIS would translate to only a ±0.1% variation in α. In acute situations, such as hyponatraemia, this variation could become more significant and could potentially bias the sensor response. The simple incorporation of a total ionic strength IPCCA sensor,[73] in

(96) Ikeda, K.; Sakamoto, Y.; Kawasaki, Y.; Miyake, T.; Tanaka, K.; Urata, T.; Katayama, Y.; Ueda, S.; Horiuchi, S. *Clin. Chem.* **1998**, *44*, 256–263.
(97) Okayama, H.; Ueda, K.; Hayaishi, O. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 1111–1115.
(98) Weith, H. L.; Wiebers, J. L.; Gilham, P. T. *Biochemistry* **1970**, *9*, 4396–4401.

tandem with the glucose sensor, would allow compensation for variations of blood ionic strength.

Reversibility of Sensing Response. A successful blood glucose sensor must sense reproducibly over extended times. To investigate the reversibility of our PCCA sensor materials, we cycled the same IPCCA through three sensing cycles over the course of 1 week, monitoring different regions. We serially characterized the response for 1, 5, and 10 mM glucose and then thoroughly washed the sensor with PBS. The sensor response is fully reversible, with the greatest variation in the average diffraction wavelength response of ±1 nm for 1 mM glucose (see Figure S-1 in the Supporting Information). This measured variation is 3 times less than the ±3 nm variation in average diffraction wavelength response observed for three separate uncycled pieces of an IPCCA exposed to 1 mM glucose. Although the absolute diffraction wavelength across one handmade IPCCA can vary by ±15 nm, the linear deformation factor (or normalized diffraction shift) within one particular sensor is nearly identical over the three sensing runs, indicating a fully reversible hydrogel sensing material.

Although the sensor response is nearly identical for the segments originating from the same original hydrogel material, these sensor materials are individually handmade and, as a result, there is imperfect reproducibility from one batch to another. We are currently working on improving the reproducibility between batches.

Practical Storage for IPCCA Sensing Material. Successful sensor commercialization requires a significant sensor shelf life. A major factor in sensor lifetime is the stability of the incorporated molecular recognition molecules. Although our IPCCA demonstrates sensing stability over ~1 week when tested directly after fabrication (Figure S-1 in the Supporting Information), longer sensor lifetimes are necessary to accommodate transportation and long-term storage. Figure S-2 in the Supporting Information shows that the response of an IPCCA to 20 mM glucose decreases by 23% after a total of ~1 month of storage in a PBS solution at room temperature. Although we have not yet diagnosed the response degradation; we speculate that it is due to a limited lifetime of the boronic acid recognition agent when stored in an aqueous environment. Therefore, we expect that storage of this material in the dry state would result in an extended shelf life.

It was previously shown that the use of filler molecules can enable reversible dehydration of hydrogels.[99−101] We recently demonstrated that Ni^{2+}-sensitive PCCA, affixed to a gel-stabilizing sheet (Bio-rad Laboratories), dried in the presence of high buffer concentrations can be reversibly rehydrated.[69]

After characterizing the sensing response of an IPCCA to various concentrations of glucose, we exposed it to a concentrated glucose solution (~45 wt %) for 30 min and then allowed it to air-dry. The hydrogel diffraction blue-shifted but continued to diffract upon drying, which indicates that the ordering of the embedded CCA was maintained during dehydration. The hydrogel was rehydrated, rinsed thoroughly in PBS, and then the sensing response was characterized. As shown in Figure 6B, the sensing ability of the material was retained, with a ~24% increase in sensitivity between glucose concentrations of 1 and 5 mM.

We attribute this increased sensitivity upon rehydration to processes which occur upon the exposure of the IPCCA to the highly concentrated glucose solution prior to dehydration. Although the total concentration of BA sites within the hydrogel remains relatively constant, the number of accessible sites is increased by this "conditioning" step. When the IPCCA is exposed to the highly concentrated glucose solution in pure water, the hydrogel swells significantly, owing to the high concentration of charged 1:1 complex sites within the hydrogel and the resulting Donnan potential. We postulate that this exercise in hydrogel volume, along with the high concentration of glucose in solution, liberates previously buried BA binding sites within the hydrogel. The increased accessibility of these additional sites results in an increased spectral response of the hydrogel sensing material after rehydration.

Using the average starting diffraction (when the glucose concentration is zero) of three separate runs for both the original IPCCA and the rehydrated IPCCA, we observe an ~8 nm decrease in initial diffraction after rehydration, which indicates that, when dried in the presence of a concentrated glucose solution, our IPCCA material reswells to ~97% of its initial volume.

The high concentration of glucose in the predrying solution prevents the irreversible and inhomogeneous collapse of the porous acrylamide hydrogel network. The excess glucose is easily removed from the material upon rehydration. The natural occurrence of glucose in bodily fluids, combined with the small required size of sensing material (1 mm × 1 mm) for optrode construction and therefore minuscule amount of glucose released upon rehydration within those fluids, makes it a logical choice for a filler molecule for use *in vivo*. The reversible rehydration of our glucose-stabilized IPCCA increases the potential for successful IPCCA commercialization.

CONCLUSIONS

We modified the synthesis and fabrication of our photonic crystal hydrogel glucose sensors to enable the monitoring of high concentrations of glucose, such as found in blood. This material is reproducibly fabricated and senses glucose reversibly over a period of 1 week. We demonstrate conditions which allow the sensor to be dried and rehydrated while retaining its diffraction and sensing properties. We significantly increased our understanding of the IPCCA sensing mechanism, by modeling the response using independently determined effective cross-link concentrations in the hydrogel as well as the independently measured glucose association constant of the 5-amino-2-fluorophenylboronic acid derivative used in the sensor.

ACKNOWLEDGMENT

We gratefully acknowledge partial financial support from BioProcessors Corporation and funding from the University of Pittsburgh.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review January 2, 2009. Accepted March 31, 2009.

AC900006X

(99) Allen, R. C. Method for Stabilizing Polyacrylamide Gels. U.S. Patent 5,159,049, October 27, 1992.

(100) Allen, R. C.; Radola, B. J. Rehydratable Polyacrylamide Gels, U.S. Patent 4,746,551, May 24, 1988.

(101) Fang, T.-Y. Reconstitutable Polyacrylamide Materials and Methods for Producing Same. U.S. Patent 5,747,600, May 5, 1998.