Photonic crystal borax competitive binding carbohydrate sensing motif†

Qingzhou Cui, Michelle M. Ward Muscatello and Sanford A. Asher*

Received 16th January 2009, Accepted 5th February 2009
First published as an Advance Article on the web 2nd March 2009
DOI: 10.1039/b901017n

We developed a photonic crystal sensing method for diol containing species such as carbohydrates based on a poly(vinyl alcohol) (PVA) hydrogel containing an embedded crystalline colloidal array (CCA). The polymerized CCA (PCCA) diffracts visible light. We show that in the presence of borax the diffraction wavelength shifts as the concentration of glucose changes. The diffraction shifts result from the competitive binding of glucose to borate, which reduces the concentration of borate bound to the PVA diols.

Introduction

There is great interest in the development of chemical sensors that can be used to visually determine the concentration of species in aqueous environments. We recently developed photonic crystal materials which can be used to construct such sensors.[1–4] The photonic crystal sensors are fabricated from highly charged monodisperse polystyrene particles synthesized *via* emulsion polymerization. These particles self-assemble into a face-centered cubic crystalline colloidal array (CCA), which diffracts visible light.[5] The CCA is embedded in an elastic hydrogel polymer matrix, forming a polymerized crystalline colloidal array (PCCA) which retains the diffraction property of the CCA.[1] Molecular-recognition groups that bind analytes selectively are attached to the PCCA.[6] Binding of the target analyte molecule causes the hydrogel volume to change which produces diffraction wavelength shifts that can be discernable to the eye.[6]

In the present study, we demonstrate a new competitive binding sensing mechanism where the analyte molecule competes for binding to a dissolved species (borate) which binds to the hydrogel backbone (poly(vinyl alcohol) (PVA) in this case). The PVA PCCA diols and added borate ions form 1 : 1 or 1 : 2 complexes with the hydrogel which alters the hydrogel volume by changing the free energy of mixing and the elastic free energy.[7] Added glucose competes for binding to borate, which changes the free borate concentration in solution which changes the concentration of borate bound to the PVA PCCA; the diffraction of the PVA PCCA depends on the glucose concentration.

The sensor developed here can be used to determine the solution concentration of molecules with diols such as carbohydrates.[8] Determination of carbohydrates is important in applications such as controlling glycemia and monitoring fermentation processes.[9–11] Although enzyme-based assays offer the most selective detection methods for carbohydrate determination,[12] only a few enzyme-based carbohydrate sensors exist.[11,13] Numerous research groups are presently developing methods to determine carbohydrates.[9,14]

Experimental

Materials

Poly(vinyl alcohol) (PVA, 88% mol hydrolyzed, MW 25 kDa) was purchased from Polysciences, Inc. 2,2-Diethoxyacetophenone (DEAP) was purchased from Acros Organics. Ion exchange resin (AG 501-X8 (D) Resin, 20–50 mesh) was purchased from Bio-Rad Laboratories, Inc. Phosphate buffered saline (PBS) was purchased from Pierce Biotechnology. D-(+)-Glucose (99.5%) was purchased from Sigma-Aldrich. Borax, HCl, NaOH, and NaCl were purchased from J. T. Baker. All chemicals were used as received.

PCCA preparation

Highly-charged, monodisperse polystyrene particles were synthesized from styrene by emulsion polymerization.[5] Synthesis of poly(vinyl alcohol) PCCA was reported previously.[15] The PVA was functionalized with glycidyl methacrylate in order to introduce vinyl groups for the PCCA photopolymerization.[16] In a typical PCCA fabrication, the modified PVA (0.25 g) was dissolved in nanopure water (1.22 g, 17.5 MΩ cm^{-1}, Barnstead Nanopure Water Purification System) *via* heating and vortexing in a 2 dram vial. The PVA solution was then cleaned with ion exchange resin (0.1 g). One milliliter of the modified PVA solution was taken from the vial after centrifuging for 5 min, and combined with the CCA (2.06 g, ~10 wt%, 118 nm diameter). Ion exchange resin (0.10 g) and photoinitiator (DEAP, 135 μL, 20% in DMSO) were added to this solution. The mixture was again vortexed and then centrifuged for another 5 min. The dispersion was injected into a cell consisting of two quartz plates, separated by a 125 μm thick Parafilm spacer. The cell was placed between two Black Ray mercury lamps (365 nm) and photopolymerized for 45 min. The cell was opened in nanopure water, and the resulting PCCA was rinsed with large quantities of nanopure water. To test the response reversibility, the PCCAs were put in 500 mL nanopure water for half an hour to wash out borate ions and glucose, and the procedure was repeated 5 times before being retested.

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA. E-mail: asher@pitt.edu; Fax: +1 412 624 0588; Tel: +1 412 624 8570

† Dedicated to Professor Seiji Shinkai on the occasion of his 65th birthday.

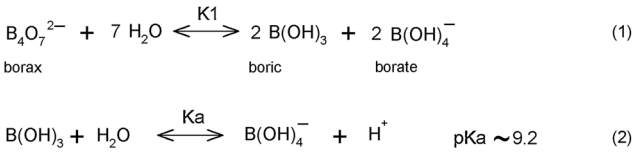
Diffraction measurement

Diffraction measurements were performed by using an Ocean Optics USB 2000-UV-VIS Spectrometer with an LS-1 Tungsten Halogen light source and an R-series fiber optic reflectance probe. A piece of the PCCA (1 cm × 1 cm) was placed in a Petri dish containing 50 mL 150 mM NaCl or 25 mM phosphate buffer solution. The PCCA was immobilized by a plastic washer which weighted it down to the bottom of the Petri dish.

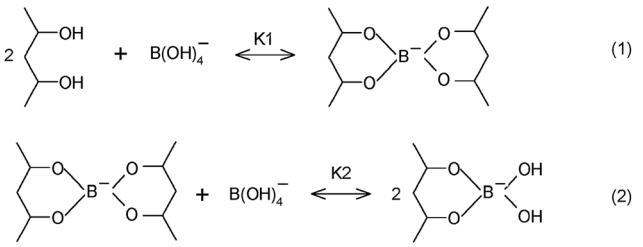
Results and discussion

Fig. 1 shows that the addition of borax (sodium tetraborate) to a 150 mM NaCl solution bathing the PVA PCCA causes the diffraction wavelength to blueshift (0 to 2 mM) and then redshift as the concentration of borax increases (>2 mM), in a manner similar to that of our previous metal cation and glucose sensing PCCAs which rely on the formation of crosslinks.[3,4] In dilute solution, borax forms boric acid and borate ions (≤25 mM, Scheme 1).[17] The borate ions reversibly bind to *cis*-1,2- or -1,3-diols to generate five-or six-membered cyclic complexes.[11,18] Scheme 2 shows that both 1 : 1 complexes and 1 : 2 crosslinking complexes can form between the borate and PVA 1,3-diols. At low borate ion concentrations where the PVA *cis*-1,3-diol groups are in excess, the 1 : 2 complexes will dominate (Scheme 2: reaction (1)). The resulting PVA crosslinks cause the diffraction to blueshift, in a manner analogous to that caused by the glucose crosslinks formed in our boronate PCCA that senses glucose[4] and by the metal cation crosslinks formed in our 8-hydroxyquinoline PCCA.[3]

As the borate concentration increases, borate forms 1 : 1 complexes with the PVA diols which break the crosslinks, resulting in diffraction redshifts (Scheme 2: reaction (2)). At borax concentrations ≥ 20 mM, the diffracted wavelength exceeds the initial diffracted wavelength in the absence of borax. Presumably, the PVA diol–borate ion complex has a more favorable free energy of mixing, which swells the hydrogel and redshifts the diffraction.



Scheme 1 Borax solution equilibria.



Scheme 2 Reactions between *cis*-diols and borate.

Boron analysis (atomic emission, R J Lee Group, Inc.) of the PVA PCCA hydrogels (3 cm × 3 cm × 125 μm), immersed in 150 mM NaCl solutions as a function of borax concentration, shows that borate selectively partitions into the hydrogel (Table 1). The PCCA boron concentration is 30-fold larger than that in the 0.5 mM borax solution reservoir. The relative enrichment decreases to 6-fold for 50 mM borax concentrations due to the saturation of borate binding within the PCCA due to the estimated PVA hydrogel diol concentration of 0.59 M (see the Appendix).

We estimated the formation constants for forming 1 : 1 and 1 : 2 complexes between PCCA *cis*-diols and borate (see Appendix).

Table 1 Boron analysis of PVA PCCA (3 cm × 3 cm × 125 μm) immersed in 150 mM NaCl solutions as a function of borax concentration

Borax concentration (mM)	0.5	2	10	50
Boron content (mM)	15	22	91	307

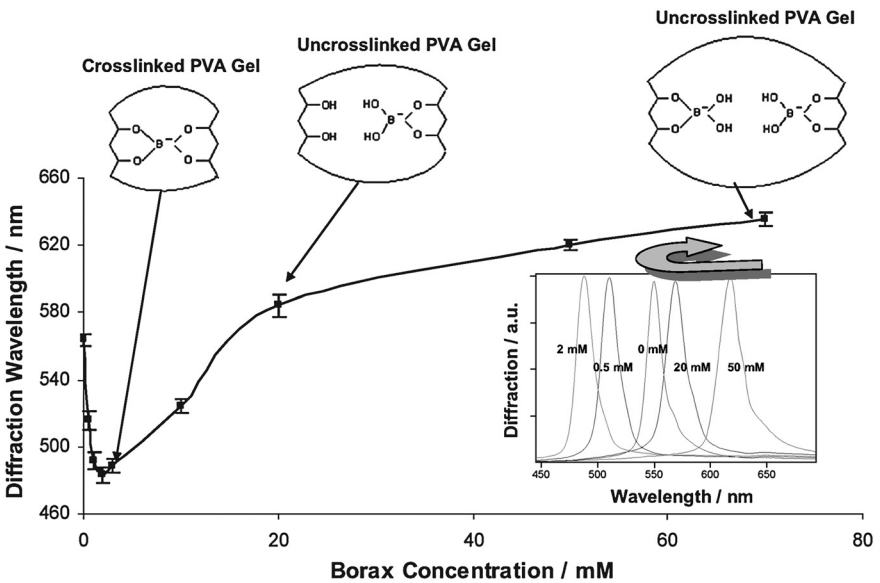


Fig. 1 Dependence of PVA PCCA diffraction on borax concentration in 150 mM NaCl solutions.

As demonstrated in the Appendix, if we assume that 1 : 2 and 1 : 1 borate–diol complexes are dominantly formed in low and high borax concentration solutions, respectively, we estimate a formation constant for the 1 : 1 complex, $K_1 = 10.9\ M^{-1}$, while for the 1 : 2 complex, $K_{21} = 62.0\ M^{-2}$. Our value of K_1 is 5-fold and K_{21} is 13-fold larger than those reported by Lorand and Edwards[19] for PVA dissolved in solution. In contrast, our K_1 is only slightly larger than that ($K_1 \approx 9\ M^{-1}$) reported by Lin *et al.*[20] It should be noted that the effective concentrations of hydroxyls in our PCCA hydrogel may be much greater than those in the solutions studied by Lorand *et al.* and Lin *et al.*

Diffraction dependence on glucose concentration

The response of the PVA PCCA to borate will depend on the presence of species such as carbohydrates in solution which can compete with the binding of PVA diols to borate. This is especially true for species like glucose with borate association constants larger than the PVA diol–borate association constant. The value of K_1 for glucose binding is 44-fold and K_{21} is 179-fold larger than those for PVA binding.[19,21] Fig. 2 shows that a bimodal PCCA diffraction response occurs due to the addition of glucose to the PVA PCCA in the presence of 10 mM borax; the PCCA diffraction wavelength blueshifts for glucose concentrations between 0 and 20 mM and then redshifts for glucose concentrations above 20 mM.

The mechanism for this bimodal response is that glucose–borate binding lowers the available uncomplexed borate ion concentration in solution. At the initial 10 mM borate concentration there is a mixture of the 1 : 2 and 1 : 1 PVA diol–borate complexes. Glucose decreases the free borate concentration, and shifts the equilibrium towards the 1 : 2 complex forming crosslinks (Scheme 3: reaction (1)). Glucose addition above 20 mM forms 1 : 1 PVA–boronate–glucose complexes which breaks the crosslinking and redshifts the diffraction (Scheme 3, reaction (2)).

These PVA PCCA photonic crystal materials can act as glucose sensors if borax is present. At high borax concentrations (20–70 mM) where hydrogel 1 : 1 borate–PVA diol complexes dominate, glucose addition causes the diffraction to blueshift (Fig. 3). The error bars were calculated from replicate runs at borax concentrations of 50 mM. In this regime, glucose addition decreases the borate concentration which decreases the PVA diol–borate 1 : 1

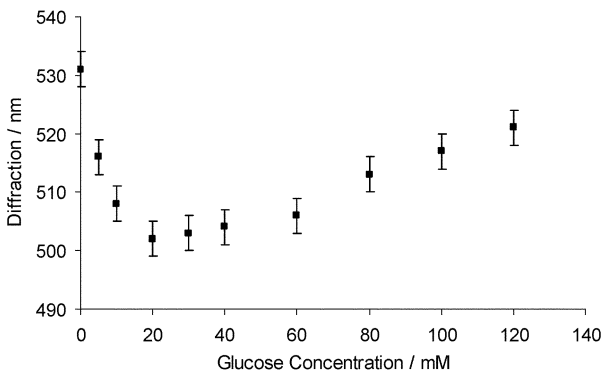


Fig. 2 Dependence of PVA PCCA diffraction on glucose in 150 mM NaCl solution at a borax concentration of 10 mM.

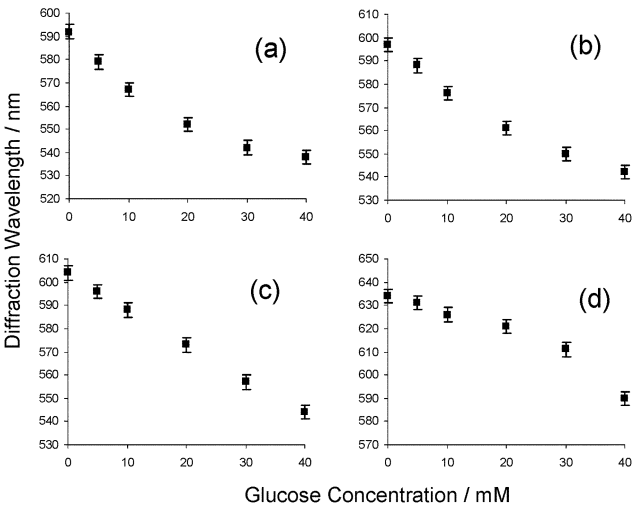
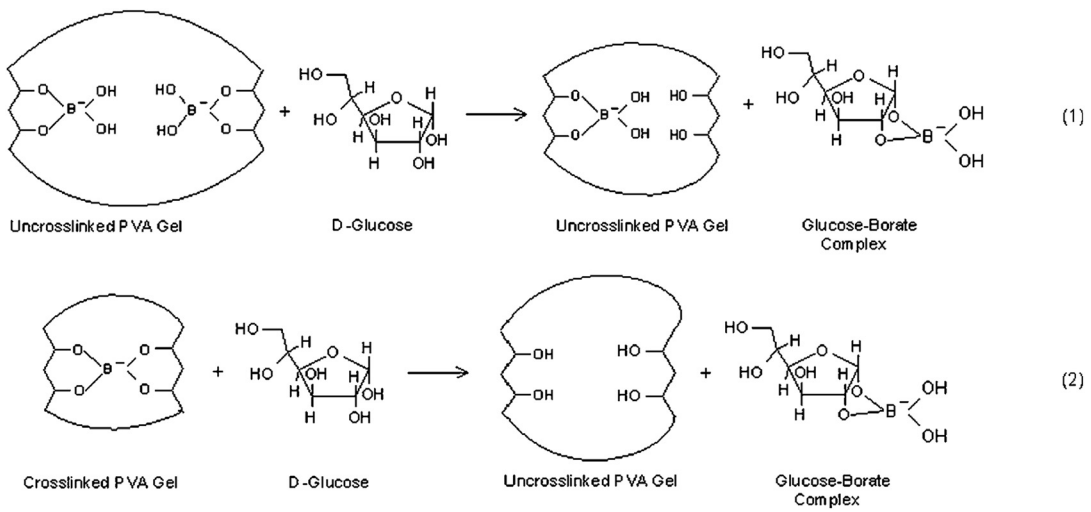


Fig. 3 Dependence of PVA PCCA diffraction on glucose in 150 mM NaCl solution at borax concentrations of (a) 20, (b) 30, (c) 50, (d) 70 mM.



Scheme 3 Competitive glucose–borate–PVA–PCCA binding equilibria.

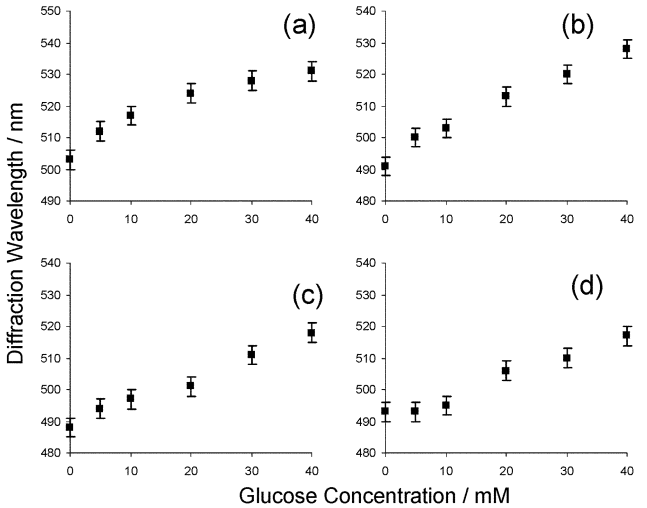


Fig. 4 Glucose dependence of PVA PCCA diffraction for 150 mM NaCl solutions at borax concentrations of (a) 0.5, (b) 1, (c) 2, (d) 3 mM.

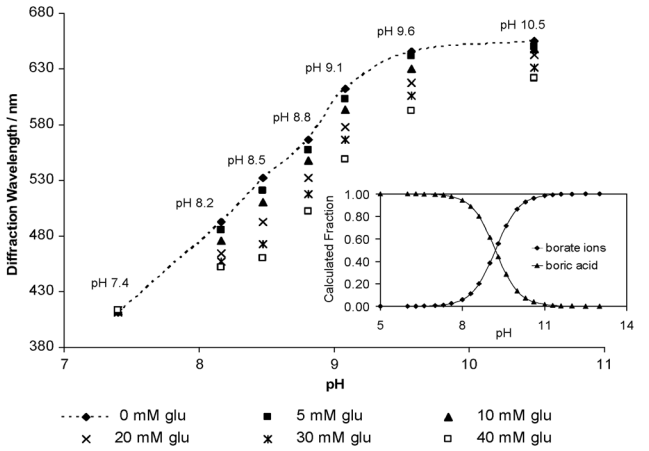


Fig. 5 Dependence of the PVA PCCA glucose response for a solution containing 25 mM sodium phosphate and 150 mM NaCl containing 50 mM borax.

complex concentration as shown by reaction (1) in Scheme 3. Boron analyses (atomic emission, R J Lee Group, Inc.) of a PVA PCCA immersed in 50 mM borax compared to a solution with 50 mM borax containing 40 mM glucose demonstrates a decrease in boron concentration from 307 to 231 mM.

In contrast, at low borax concentrations, where 1 : 2 borate–PVA diol crosslink complexes dominate, glucose addition decreases the free borate concentration resulting in a decrease in the number of crosslinks as shown by Fig. 4 which demonstrates that the diffraction redshifts upon the addition of glucose to solutions containing 0.5, 1, 2, and 3 mM of borax. The curvature in Fig. 3 and Fig. 4 occurs because the impact of glucose differs between different concentration regimes. For example, in Fig. 3a, the initial glucose addition significantly decreases the 1 : 1 borate–diol complex concentration within the PCCA. However, further glucose addition has little impact at low borate–diol complex concentrations.

The reproducibility in reading the diffraction appears to be the major error in the measurement. The magnitude of this error, $3\sigma \approx$ 0.6 nm, allows us to estimate a glucose detection limit of 0.33 mM for 50 mM borax solution containing 150 mM NaCl (pH = 8.5).

pH dependence of the glucose measurement

We examined the pH dependence of the glucose response for 50 mM borax solutions (Fig. 5). The PCCA swelling signaled by the diffraction redshift with increasing pH results from the increased concentration of 1 : 1 borate–PVA complexes as the borate concentration increases. We originally expected that boric acid would show a $pK_a = 9.1$–9.2[22] which would give rise to the pH dependence of boric acid and borate concentrations shown in the inset to Fig. 5. The line drawn through the Fig. 5 zero glucose pH dependent diffraction data shows a titration behavior suggestive of a process with an effective pK_a for boric acid within the hydrogel decreased to ~8.4. The origin of this shift in effective pK_a results from the saturation of the binding of borate to the hydrogel. As the concentration of borate exceeds that of the PVA diols the binding of borate saturates and the slope of the diffraction redshift decreases.

Table 2 Boron analysis for the PVA PCCAs (3 cm × 3 cm × 125 μm) immersed in 150 mM NaCl solutions with 50 mM borax as a function of pH values

pH	7.4	8.4	9.2	10.1
Boron content (mM)	155	231	307	292

At pH 7.4 we expect and observe that there is little response of the PVA PCCA to glucose because the boric acid present binds glucose with a small affinity, much less than that of borate.[23] As expected, the response increases with pH until pH = 8.5 where it starts to drop. The glucose dependence results from competition of glucose for binding to the borate ions. Boron analysis of dried PVA hydrogels, immersed in 50 mM borax solutions, shows that the boron content increases with increasing pH from 7.4 to 9.2 where it saturates (Table 2).

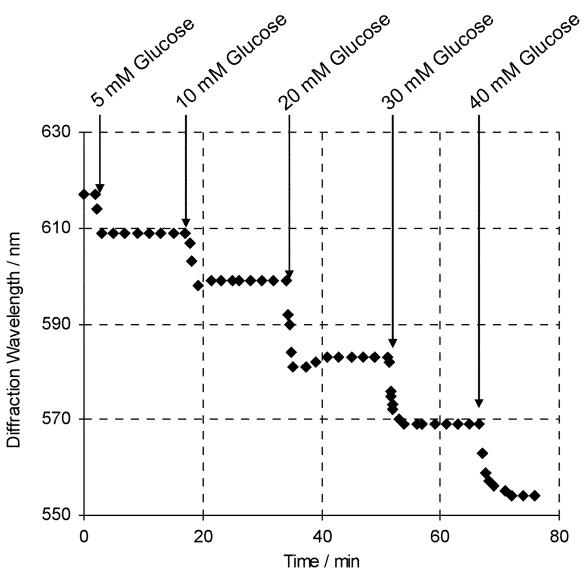


Fig. 6 Response kinetics of the PVA PCCA glucose sensor upon exposure to multiple additions of a freshly prepared glucose stock solution.

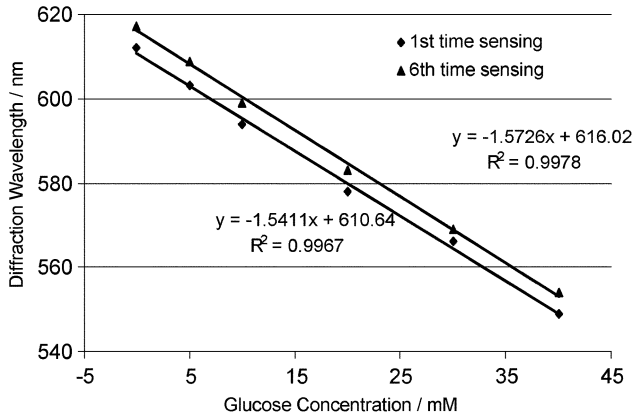


Fig. 7 Comparison of initial diffraction response of the PVA PCCA sensor containing 50 mM borax to glucose and after five glucose sensing measurements at pH 9.08, which were followed by washing with a pH 7.4 25 mM sodium phosphate and 150 mM NaCl solution. The sensitivity does not change, but there is a ~5 nm diffraction redshift.

As the borate concentration increases above that of the PVA diols at pH values above 8.5 the response to glucose decreases because we are already in a saturating borate–PVA binding regime (Fig. 5). A similar result is observed in Fig. 3d which occurs at the highest borax concentrations.

Reaction kinetics and response reversibility

Fig. 6 shows that these PVA PCCA sensors respond in real time to glucose and that their diffraction wavelength shifts complete within 2–3 minutes after glucose addition. The PVA PCCA sensor response is both reversible and reproducible. Fig. 7 compares the initial sensing response of a PVA PCCA compared to that occurring after five replicate sensing runs where the sensor was washed between sensing runs with 150 mM NaCl solutions. The glucose sensitivity is unchanged but we see a ~5 nm redshift in the sensor diffraction.

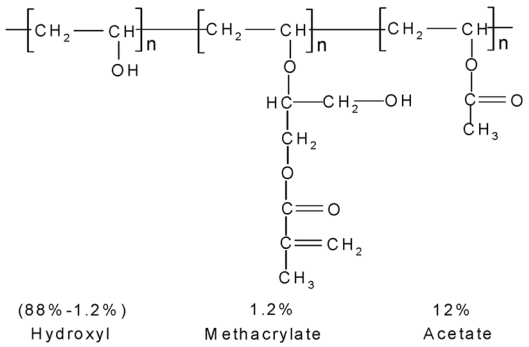
These PVA PCCAs have a major advantage over our classic acrylamide PCCAs in their ability to be reversibly dehydrated.[15] When rehydrated they show similar sensitivity to that prior to dehydration. This enables storage of the sensors for later use.

Conclusions

We developed a competitive binding motif for use in photonic crystal PCCA sensing. We utilize a PVA PCCA and add borate to the solution which binds to the PVA hydrogel, crosslinking it as well as increasing the hydrogel polymer solubility. We demonstrate sensing of glucose which results from the competitive binding between the glucose and the borate which decreases the borate binding to the PVA PCCA. The glucose sensing is reversible and the diffraction shifts occur in real time (3 min). The sensors can be dehydrated for long-term storage.

Appendix: hydroxyl density and borate formation constants

The PVA for making the hydrogel is 88 mol% hydrolyzed, where 1.2% of the side chains are methacrylated according to NMR data. Thus the PVA molecular structure is:



The weight percentage of hydroxyl groups is 29.2% in pure PVA. From the chemical composition used we calculate that the hydroxyl concentration of the PCCA hydrogel is 1.18 M. This would suggest a 0.59 M concentration of *cis*-1,3-diols. A PCCA sample (1cm × 1cm × 125 μm) would contain a total of 7.5 μmole *cis*-1,3-diols. The number of moles of PCCA *cis*-diol groups is, thus, small compared to the amount of borate ion in solution. This ensures that there will be no depletion of the borate concentration in the reservoir.

We can estimate the association constants for the borate with the PVA PCCA. Boric acid has the following acid–base equilibrium:

$$\mathrm{BH} \xleftrightarrow{K_a} \mathrm{B} + \mathrm{H}^+ \qquad K_a = \frac{[\mathrm{B}][\mathrm{H}^+]}{[\mathrm{BH}]} \tag{1}$$

where BH is boric acid, B is the borate ion.

Within the hydrogel, borate ions form 1 : 1 and 1 : 2 complexes with the PVA *cis*-diol groups:

$$\mathrm{B} + \mathrm{L} \xleftrightarrow{K_1} \mathrm{BL} \qquad K_1 = \frac{[\mathrm{BL}]}{[\mathrm{B}][\mathrm{L}]} \tag{2}$$

$$\mathrm{B} + 2\mathrm{L} \xleftrightarrow{K_{21}} \mathrm{BL_2} \qquad K_{21} = \frac{[\mathrm{BL_2}]}{[\mathrm{B}][\mathrm{L}]^2} \tag{3}$$

where L is a PVA diol group.

For a borax concentration ≤ 25 mM, where borax is completely dissociated into borate ions and boric acid, the borate ion concentration can be determined from the known association constant K_a and the solution pH.

At a 0.5 mM borax concentration, the 1 : 2 complexes appear to dominate as evidenced by the diffraction blue shift. The boron analysis (Table 1) shows that boron content is 15 mM within the hydrogel. Assuming that all borate ions form 1 : 2 complexes, $[BL_2] = 15$ mM. Since $[L] = [L]_{total} - 2[BL_2]$ and [B] can be calculated from eqn (1) with a pK_a of 9.2. Thus,

$$K_{21} = \frac{[\mathrm{BL_2}]}{[\mathrm{B}][\mathrm{L}]^2} = 62.0\ \mathrm{M}^{-2}$$

At 10 mM borax concentrations, 1 : 1 complexes dominate and boron analysis shows that the boron concentration within the hydrogel is 91 mM. Assuming that all borate ions form 1 : 1 complexes, $[BL] = 91$ mM. Since $[L] = [L]_{total} - [BL]$ and [B] is known from the boric acid equilibrium (eqn (1)). Thus,

$$K_1 = \frac{[\mathrm{BL}]}{[\mathrm{B}][\mathrm{L}]} = 10.9\ \mathrm{M}^{-1}$$

Acknowledgements

We gratefully acknowledge financial support from NIH Grant 2R01 EB004132.

References

1 J. H. Holtz and S. A. Asher, *Nature*, 1997, **389**, 829.
2 K. W. Kimble, J. P. Walker, D. N. Finegold and S. A. Asher, *Anal. Bioanal. Chem.*, 2006, **385**, 678; S. A. Asher, S. F. Peteu, C. E. Reese, M. X. Lin and D. Finegold, *Anal. Bioanal. Chem.*, 2002, **373**, 632; V. L. Alexeev, S. Das, D. N. Finegold and S. A. Asher, *Clin. Chem.*, 2004, **50**, 2353; M. Ben-Moshe, V. L. Alexeev and S. A. Asher, *Anal. Chem.*, 2006, **78**, 5149.
3 S. A. Asher, A. C. Sharma, A. V. Goponenko and M. M. Ward, *Anal. Chem.*, 2003, **75**, 1676.
4 V. L. Alexeev, A. C. Sharma, A. V. Goponenko, S. Das, I. K. Lednev, C. S. Wilcox, D. N. Finegold and S. A. Asher, *Anal. Chem.*, 2003, **75**, 2316.
5 C. E. Reese, C. D. Guerrero, J. M. Weissman, K. Lee and S. A. Asher, *J. Colloid Interf. Sci.*, 2000, **232**, 76.
6 S. A. Asher, V. L. Alexeev, A. V. Goponenko, A. C. Sharma, I. K. Lednev, C. S. Wilcox and D. N. Finegold, *J. Am. Chem. Soc.*, 2003, **125**, 3322.
7 P. J. Flory, *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, New York, 1953.
8 A. D. McNaught, *Pure Appl. Chem.*, 1996, **68**, 1919.
9 K. Aslan, J. Zhang, J. R. Lakowicz and C. D. Geddes, *J. Fluoresc.*, 2004, **14**, 391; C. Cannizzo, S. Amigoni-Gerbier and C. Larpent, *Polymer*, 2005, **46**, 1269; T. D. James and S. Shinkai, *Top. Curr. Chem.*, 2002, **218**, 159.
10 D. C. Klonoff, *Diabetes Care*, 2005, **28**, 1231; J. C. de Menezes, S. da Silva Alves, J. M. Lemos and S. F. de Azevedo, *Biotechnol. Tech.*, 1992, **6**, 1; G. L. Cote and B. D. Cameron, *J. Biomed. Opt.*, 1997, **2**, 275; J. Myung, K. B. Kim and C. M. Crews, *Med. Res. Rev.*, 2001, **21**, 245; W. Yang, S. Gao, X. Gao, V. V. R. Karnati, W. Ni, B. Wang, W. B. Hooks, J. Carson and B. Weston, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2175.
11 T. D. James, K. R. A. Sandanayake Samankumara and S. Shinkai, *Angew. Chem., Int. Ed.*, 1996, **35**, 1911.
12 R. Jelinek and S. Kolusheva, *Chem. Rev.*, 2004, **104**, 5987; R. P. Baldwin, *J. Pharm. Biomed. Anal.*, 1999, **19**, 69.
13 T. D. James, P. Linnane and S. Shinkai, *Chem. Commun.*, 1996, 281.
14 O. A. Sadik and F. Yan, *Anal. Chim. Acta*, 2007, **588**, 292; F. He, K. Chen, J. Fu, L. Nie and S. Yao, *Anal. Sci.*, 1995, **11**, 1001.
15 M. M. Ward Muscatello and S. A. Asher, *Adv. Funct. Mater.*, 2008, **18**, 1186.
16 F. Cavalieri, F. Miano, P. D'Antona and G. Paradossi, *Biomacromolecules*, 2004, **5**, 2439; P. Martens and K. S. Anseth, *Polymer*, 2000, **41**, 7715.
17 N. Ingri, G. Lagerstrom, M. Frydman and L. G. Sillen, *Acta Chem. Scand.*, 1957, **11**, 1034; M. Maeda, T. Hirao, M. Kotaka and H. Kakihana, *J. Inorg. Nucl. Chem.*, 1979, **41**, 1217.
18 J. M. Sugihara and C. M. Bowman, *J. Am. Chem. Soc.*, 1958, **80**, 2443; Y. Ma, L. Qian, H. Huang and X. Yang, *J. Colloid. Interface Sci.*, 2006, **295**, 583; H. D. Smith, Jr. and R. J. Wiersema, *Inorg. Chem.*, 1972, **11**, 1152; S. Chapelle and J. F. Verchere, *Tetrahedron*, 1988, **44**, 4469.
19 J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769.
20 H.-L. Lin, W.-H. Liu, Y.-F. Liu and C.-H. Cheng, *J. Polym. Res.*, 2002, **9**, 233.
21 G. L. Roy, A. L. Laferriere and J. O. Edwards, *J. Inorg. Nucl. Chem.*, 1957, **4**, 106.
22 H. B. Davis and C. J. B. Mott, *J. Chem. Soc., Faraday Trans.*, 1980, **76**, 1991; R. Aruga, *J. Chem. Soc., Dalton Trans.*, 1988, 2971; M. Bishop, N. Shahid, J. Yang and A. R. Barron, *Dalton Trans.*, 2004, 2621; J. A. Tossell, *Geochim Cosmochim Acta*, 2005, **69**, 5647.
23 M. Shibayama, M. Sato, Y. Kimura, H. Fujiwara and S. Nomura, *Polymer*, 1988, **29**, 336; E. Pezron, A. Ricard, F. Lafuma and R. Audebert, *Macromolecules*, 1988, **21**, 1121; E. Ivanov, H. Larsson, Y. Galaev and B. Mattiasson, *Polymer*, 2004, **45**, 2495; H. Ochiai, S. Shimizu, Y. Tadokoro and I. Murakami, *Polymer*, 1981, **22**, 1456.

