



Color changing block copolymer films for chemical sensing of simple sugars

Omar B. Ayyub^a, Jennifer W. Sekowski^b, Ta-I Yang^a, Xin Zhang^c, Robert M. Briber^c, Peter Kofinas^{a,*}

^a Fischell Department of Bioengineering Room 2330 Jeong H. Kim Engineering Building, University of Maryland, College Park, MD 20742, USA

^b U.S. Army Edgewood Chemical Biological Center, RDCB-DRB-C, 5183 Blackhawk Rd., Aberdeen Proving Ground, MD 21010-5424, USA

^c Department of Materials Science and Engineering, University of Maryland, College Park, MD 20740, USA

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ABSTRACT

We investigated the use of functionalized photonic block copolymer films for the detection of glucose. Polystyrene-*b*-poly(2-vinyl pyridine) (PS-*b*-P2VP) block copolymers were chemically functionalized with 2-(bromomethyl)phenylboronic acid and cast into films that reflect a visible color when exposed to aqueous media. The 2-(bromomethyl)phenylboronic acid functionality can reversibly bind to glucose. When exposed to high concentrations of glucose the polymer responded with a red shift in color. Low concentration exposure of glucose caused the polymer films to blue shift in color. The BCP films also exhibited a selective response to fructose, mannose or galactose, giving a different response depending on which sugar is present. The color of the polymer was tuned to blue, green, yellow or orange by varying the film's crosslink density. The color change can be visually observed without the use of equipment such as a spectrometer.

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1. Introduction

Chemical sensors have been developed from a wide array of materials that can change color upon exposure to a target molecule. These sensor systems are generally based on photonic crystals that have been modified to recognize the target molecule. The photonic crystal contains periodic nanostructures with differing indices of refraction that interact with visible light. The material can be functionalized to recognize or bind to specific chemical targets. Recognition of the target will alter the spacing of the periodic nanostructure thereby changing the way it interacts with visible light. The advantage of such systems lies in producing a discernable change in color. This concept has been applied in the fabrication of colloidal hydrogel systems, porous silicon systems as well as through the use of lithography techniques on other substrates. Asher et al. demonstrated the use of a photonic crystal polymerized colloidal hydrogel system (Cui et al., 2009; Lee and Asher, 2000). Such material could act as a sensor for glucose but required the complex process of synthesizing monodisperse, highly charged polystyrene particles. Porous silicon (Lee and Fauchet, 2007; Li et al., 2003) and nanoprint lithography (Endo et al., 2010) have also been reported as photonic crystal chemical sensing platforms. These systems

can be problematic in producing a discernable response without analytical measurements. Therefore, a sensor that can be easily fabricated and yield an instantaneous, visibly discernable response is needed.

One of the material systems investigated for its use as photonic crystals is self-assembled microphase separated layers in block copolymer films (Edrington et al., 2001; Kang et al., 2007; Urbas et al., 2000). Block copolymers (BCPs) consist of two or more chemically distinct sequences of monomer repeat units linked together through a covalent bond. Upon evaporation from a solvent, BCPs will microphase separate into solid films displaying a number of different morphologies (e.g. hexagonal, cubic, gyroid, lamellar) depending on the relative volume fraction of each block (Bates and Fredrickson, 1990). BCPs in which both blocks are of equal molecular weight generally exhibit the lamellar morphology. Self-assembly into a lamellar morphology is significant in producing a BCP photonic crystal. If there is enough contrast in refractive index between the two blocks in the lamellar structure then certain wavelengths of light will be reflected by the material. This phenomenon is dictated by:

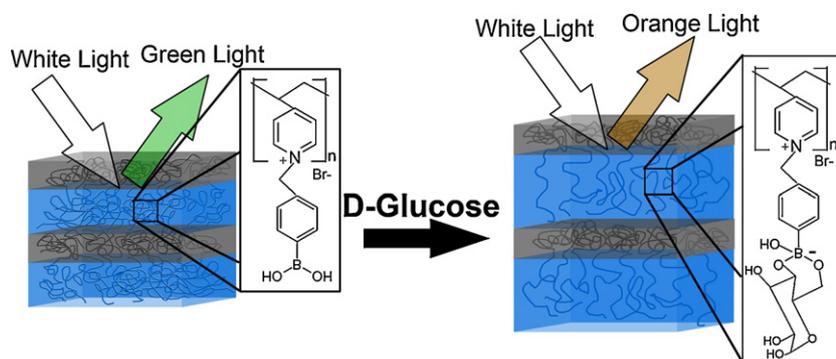
$$\lambda_1 = 2(n_1d_1 + n_2d_2)$$

where λ_1 is the reflected wavelength, n_i is the refractive index of layer i and d_i is the thickness of layer i .

In this study, the diblock copolymer polystyrene-*b*-poly(2-vinylpyridine) (PS-*b*-P2VP), which microphase separates into a lamellar periodic stack, was explored for its use as a chemical sensor to detect and respond to glucose with a change in color. The 2-vinyl pyridine (P2VP) block was quaternized

* Corresponding author at: Fischell Department of Bioengineering, 1120 Jeong H. Kim Engineering Building 225, University of Maryland, College Park, MD 20742, USA. Tel.: +1 301 405 7335.

E-mail address: kofinas@umd.edu (P. Kofinas).



Scheme 1. Schematic depicting the BCP film color change. Initially the BCP film is in deionized water. The positive charge on the pyridine allows the P2VP block to swell in the water. This gives the BCP film sufficient thickness to reflect visible light, in this case green light. When D-glucose is introduced it binds to the phenylboronic acid functionality which lowers the pKa of the boronic acid forming the negatively charged boronate complex. The additional negative charge swells the BCP film causing it to reflect light in the wavelength of orange.

with 2-(bromomethyl)phenylboronic acid, which placed a positive charge on the pyridine ring of the block. This charge allows the BCP film to swell in aqueous media. The swelling changes the thickness of the block allowing it to interact with wavelengths of visible light. Kang et al. (2007) have reported lamellar PS-b-P2VP films quaternized with bromoethane, which places a positive charge in the P2VP block, and attaches an ethyl group to the nitrogen atom. In our work, the quaternizing agent used contributes a boronic acid residue, giving the BCP the ability to bind to sugars such as glucose. We hypothesized that binding would induce a change in the distance between the lamellae causing a change in the wavelength light reflected by the polymer, thus allowing the BCP to act as a glucose sensor.

Boronic acids have been of great interest in chemical sensing due to their ability to covalently bind to sugar molecules such as glucose (Bosch et al., 2004; Chen et al., 2009; Kim et al., 2007). Although sensing glucose has applications in diabetic medicine our decision to explore glucose sensing using the boronic acid functionalized BCP system, was designed to serve as a model system to test the concept that block copolymer based photonic crystals can be fabricated to act as chemical sensors for small molecule detection. In the work described in this manuscript, the polymer film was tested for successful attachment of the boronic acid, retention of the lamellar morphology post-functionalization, and sensitivity and specificity to simple sugar binding.

A schematic of the BCP film sensor can be seen in Scheme 1. Initially the BCP film is fabricated to exhibit a periodic lamellar stack. The P2VP block of the block copolymer is functionalized with phenylboronic acid placing a positive charge on the pyridine ring, which allows the P2VP block to swell in aqueous media until its thickness is large enough to interact with visible light, in this case reflecting green light. The phenylboronic acid can bind sugars and will do so when exposed to a glucose solution. As shown in Scheme 1 the boronic acid binds to the 1,3 diol functionality. It has been reported that this is the kinetically favored binding site on glucose as well as the 1,2 cis diol (Bielecki et al., 1999). This binding event lowers the pKa of the phenylboronic acid causing it to form the negatively charged boronate complex. The negative charge triggers additional swelling of the BCP film changing its color from green to orange. We have shown that after functionalization the BCP film can respond to a glucose solution and shows a selective response when exposed to different sugars such as fructose, mannose or galactose.

2. Methods

2.1. Fabrication of photonic BCP films

The procedure for fabrication of the PS-b-P2VP films was adapted from Kang et al. (2007). The PS-b-P2VP block copolymers were purchased from Polymer Source (Montreal, Canada). The molecular weight of each block of the copolymer was 133,000 g/mol. A 5% weight/volume stock solution of PS-b-P2VP was prepared in propylene glycol monomethyl ether acetate (PGMEA). The films were prepared by spin-casting 300 μ l of the PS-b-P2VP solution onto 1'' \times 1'' glass slides at 350 rpm for 2 min. The glass slides were purchased from Ted Pella (Redding, CA), and were functionalized with 3-(aminopropyl)triethoxysilane. The spin-cast block copolymer films were subsequently annealed in chloroform vapor at room temperature for 24 h to allow them to self-assemble into a lamellar structure.

2.2. Functionalization and tuning of photonic properties

The P2VP block of the spin-cast films was quaternized with 2-(bromomethyl)phenylboronic acid. The quaternization reaction places a phenylboronic acid functional group in the P2VP block, allowing the polymer to bind sugars such as glucose. The quaternization reaction was carried out by immersing the spin-cast, annealed block copolymer films in a solution of 40 mg of 2-(bromomethyl)phenylboronic acid in 40 ml of acetonitrile and allowing it to reflux for 5 h. The quaternized polymers were then removed from the solution and dried in a 50°C oven for 1 h. To tune the optical properties of BCP film a crosslinking agent, 1,4-dibromo-2-butanol, was introduced into the acetonitrile solution. Different molar ratios of 2-(bromomethyl)phenylboronic acid to 1,4-dibromo-2-butanol were tested at 0.05 mmol as the total monomer amount. The same protocol was used to investigate the effect of different degrees of crosslinking on the color of the BCP films.

2.3. Characterization

Fourier transform infrared spectroscopy (FTIR) was utilized to determine whether the boronic acid functionality was successfully attached. The functionalized polymer film was removed from the glass slide by immersion in a 5%v/v aqueous solution of hydrofluoric acid. The freestanding polymer film was dried for 24 h in a 50°C oven. An FTIR spectrum was obtained of the functionalized polymer film using a Thermo Nicolet FT-Raman, Model 670. To determine

whether the lamellar morphology was maintained after functionalization, images of the polymer were taken using transmission electron microscopy (TEM). The sample was prepared by embedding a section of the functionalized polymer film in Spurr's resin purchased from Ted Pella. The embedded polymer film was then dry microtomed and stained through exposure to solid iodine for 1 h. The images were taken using a JEM-2100F 200 kV transmission electron microscope.

2.4. Sugar detection

Once the films were functionalized with 2-(bromomethyl)phenylboronic acid, they were exposed to various concentrations of D-glucose to observe if the films would respond with a color change. Aqueous solutions of 50, 40, 30, 10, 5 and 1 mg/ml of D-glucose were prepared. The films were soaked in 20 ml of DI water and a coverslip was placed on top of the polymer film on the glass slide to retain the water in the film as it was transferred to the UV-Visible spectrometer. The water-swollen control sample was then placed in a Perkin Elmer Lambda25 UV-Visible spectrometer and the visible spectrum was measured. Each film was then soaked in one of the prepared glucose concentrations and the visible spectrum was again measured and compared to the film soaked in pure water. To test for selectivity between glucose and other sugars, films were prepared and exposed to 50 mg/ml solutions of glucose, fructose, galactose and mannose and their visible spectra measured.

3. Results and discussion

3.1. Characterization

In order for the BCP film to diffract light towards the observer, the lamellar morphology must be oriented parallel to the glass substrate. The 3-(aminopropyl)triethoxysilane functionalization on the glass substrate interacts with the P2VP block of the BCP by influencing the morphology to be parallel to the substrate. The BCP film is then annealed by exposing it to chloroform vapor, mobilizing the polymer chains and allowing them to form the parallel lamellae. This annealing process is very specific, requiring precise conditions to be successful. Environmental factors such as room humidity and evaporation rate of the annealing solvent can cause the BCP film to have poor morphology or break the interactions between the BCP and the substrate. It was found that low humidity, approximately 20%, and relatively slowed evaporation rate of the chloroform dramatically improved morphology. After completion of the annealing process the BCP film is chemically functionalized by attaching 2-(bromomethyl)phenylboronic acid to the P2VP block. This chemical modification requires that the BCP film be exposed to acetonitrile at an elevated temperature. Such harsh conditions could potentially damage the BCP film or disrupt its delicate lamellar morphology. If the lamellar morphology is disrupted by the chemical modification, then the photonic properties of the BCP film will be diminished. TEM was utilized to determine whether the lamellar morphology was maintained after functionalization with the 2-(bromomethyl)phenylboronic acid. Fig. 1 presents the electron micrograph of the cross section of both an unmodified BCP film and of a BCP film functionalized with 2-(bromomethyl)phenylboronic acid. As seen in Fig. 1 the lamellar morphology needed for the film's optical properties is maintained after functionalization of the P2VP block. In the unmodified film of Fig. 1A, the layer thickness of the P2VP block and PS block are similar in size as the molecular weight of each block is comparable. The iodine-stained block is the P2VP. After chemical functionalization, as seen in Fig. 1B, the lamellar morphology is maintained however; there is an apparent increased thickness of the darker iodine stained P2VP block versus

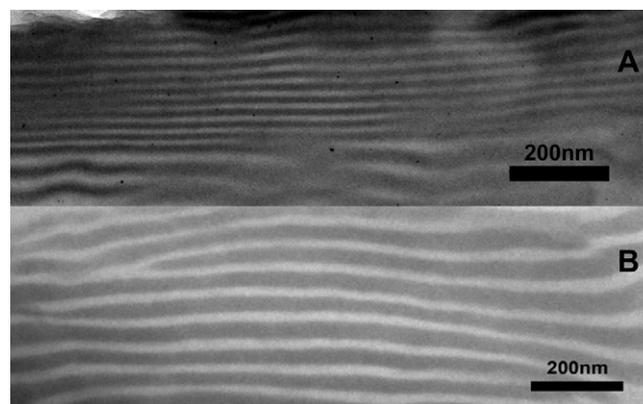


Fig. 1. (A) TEM of annealed, unmodified PS-b-P2VP film. (B) TEM of PS-b-P2VP film functionalized with 2-(bromomethyl)phenylboronic acid exhibiting a lamellar morphology. The darker iodine stained P2VP block appears thicker due to the increased mass of the P2VP block from the functionalization as compared to the cross section seen in the unmodified film, A.

the lighter PS block due to the increased mass added to the P2VP block from the 2-(bromomethyl)phenylboronic acid.

Verification that the PS-b-P2VP films were functionalized was obtained by analyzing the chemical structure with FTIR. The pyridine group of the P2VP block substitutes the bromine in the 2-(bromomethyl)phenylboronic acid which covalently bonds the boronic acid functionalization to the pyridine. The boronic acid functionalization places a positive charge on the nitrogen atom in the pyridine ring of the P2VP block as the pyridine groups are converted to pyridinium. This conversion from pyridine to pyridinium can be observed using FTIR. The FTIR spectrum of a modified and unmodified film was taken. A peak appeared at 1627 cm^{-1} in the modified film indicating the conversion of pyridine to pyridinium, or the placement of a formal positive charge on the nitrogen atom of the pyridine ring. This functionalization serves two important purposes. The first of which is to place a formal positive charge on the P2VP block of the BCP film allowing it to swell in water. This swells the P2VP block to a large enough thickness, which allows it to interact with visible light as dictated by Bragg's law. The second purpose of the functionalization is to introduce boronic acid to the BCP film. Boronic acid binds to sugar molecules allowing the BCP film to recognize and respond to simple sugars with a change in color. This substitution reaction was relatively easy to perform requiring exposure of the BCP film to a solution of 2-(bromomethyl)phenylboronic acid in acetonitrile while refluxing for 5 h. This methodology could potentially open up other avenues for different chemical functionalizations each with their own sensing application.

3.2. Tuning the polymer film photonic properties

Demonstrating control over the optical properties or color of the BCP film is vital for producing a reliable chemical sensor. To tune the color of the functionalized BCP film, varying degrees of crosslinking were introduced into the P2VP block. The crosslinker used was 1,4-dibromo-2-butanol in conjunction with the boronic acid functionalization. The BCP was exposed to various molar ratios of 1,4-dibromo-2-butanol (crosslinker) to 2-(bromomethyl)phenylboronic acid (quaternerizer). Increasing or decreasing the mole fraction of the crosslinker, allows the collapsing or swelling the polymer film in water, which tunes the color of the BCP.

The BCP films can be fabricated to exhibit different colors by controlling the swelling of the polymer film through variations in crosslink density. The BCP films have successfully been tuned to

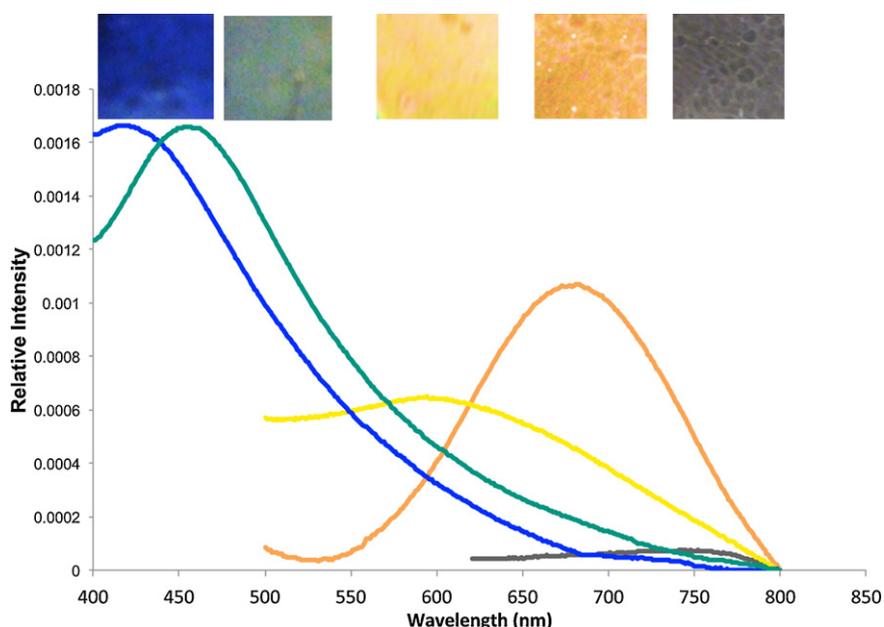


Fig. 2. The BCP film color can be tuned to blue, green, yellow, orange or transparent (infrared) colors depending on the degree of crosslinking. Increasing the crosslink density inhibits swelling which blueshifts the color of the polymer film. As seen each BCP film displays a different color and visible spectra depending on the molar ratio of crosslinker to quaternizer seen in Supplementary Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

blue, green, yellow, orange or infrared colors. Fig. 2 shows each of the colored BCP films and their corresponding visible spectra. As seen the BCP film's color is intense and easy to recognize by eye. There is no need for extraneous equipment to distinguish one color from another. This allows the simple fabrication sensor to be utilized by individuals who would not need specific training to determine if the BCP film has responded to a specific analyte.

3.3. Response to glucose

Boronic acid can bind to 1,2 and 1,3 cis diols, a chemical functionality commonly found in sugar molecules. The binding of a sugar to the boronic acid lowers its pKa. The pKa change increases the number of boronic acid residues that form the boronate complex, which is negatively charged. Such negative charge then causes the BCP film to swell, thereby changing its color. The PS-b-P2VP films functionalized with 2-(bromomethyl)phenylboronic acid were exposed to a range of concentrations of aqueous D-glucose solutions. The tested polymer films were initially green in pure deionized water due to the functionalization placing a positive charge on the P2VP block. The green films exposed to the 50 mg/ml glucose solution instantly swelled and became orange in color. This shift in color is visible to the eye and can be detected without the use of equipment such as spectrometer. This color change and UV-Visible spectrum can be seen in Fig. 3.

UV-Vis spectroscopy was used to quantify the films' exposure to a range of aqueous concentrations of glucose (0–50 mg/ml). It was predicted that the spectrum peak should redshift after exposure to glucose due to the binding and incorporation of the glucose molecule causing swelling. When exposed to concentrations of 50 mg/ml and 40 mg/ml glucose, the films shifted from green to orange or yellow, respectively as seen in Fig. 4. This indicates that they became swollen as expected. However, exposure to lower concentrations of glucose had a very different effect.

As seen in Fig. 4, after exposure to concentrations of 30, 10 and 5 mg/ml glucose, the films blueshifted, indicating that the polymer film had collapsed when exposed to these concentrations of glucose solution. Interestingly, this counterintuitive observation of swelling at high concentrations and collapsing at lower

concentrations has been reported in similar boronic acid based detection systems (Cui et al., 2009; Lee and Asher, 2000). Asher et al. demonstrated a similar bi-modal response in a polymerized crystalline colloidal hydrogel system. Their group reported that when their hydrogel was exposed to high concentrations of glucose each boronic acid functionality bound to one glucose molecule, placing a negative charge on the boron atom, which caused the film to swell

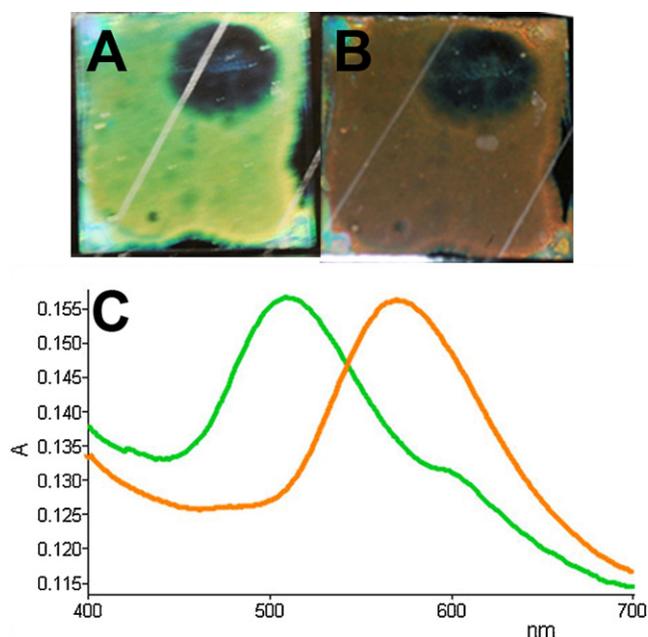


Fig. 3. (A) When exposed to pure water the film swelled due to the positive charge placed on the P2VP block from the functionalization. (B) Once immersed in a glucose solution the functionalized polymer film swelled further due to the boronic acid groups binding glucose. When bound to glucose, a negative charge exists on the boron atom of the boronic acid moiety causing the film to further swell and become orange in color. (C) The UV-Visible spectrum of the functionalized film in both water and glucose solution. In water the film swells to reflect a peak wavelength of 510 nm corresponding to the color green. In the glucose solution, the film swells and reflects a peak wavelength of 590 nm corresponding to orange.

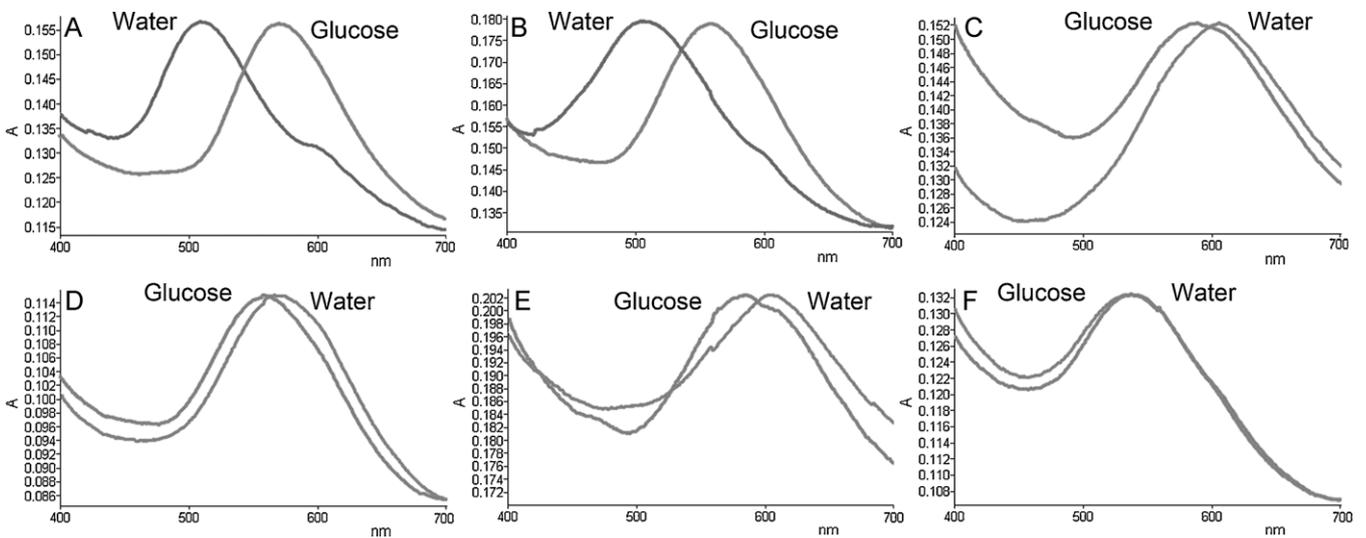


Fig. 4. The shift in peak wavelength of each BCP sample immersed in various concentrations of glucose solution of (a) 50 mg/ml, (b) 40 mg/ml, (c) 30 mg/ml, (d) 10 mg/ml, (e) 5 mg/ml, and (f) 1 mg/ml. 50 and 40 mg/ml solutions caused an increase, or redshift, in wavelength indicating that the polymer film had swelled. However, a decrease, or blueshift, was observed in the BCP samples exposed to 30, 10, and 5 mg/ml solutions, indicating that the film collapsed.

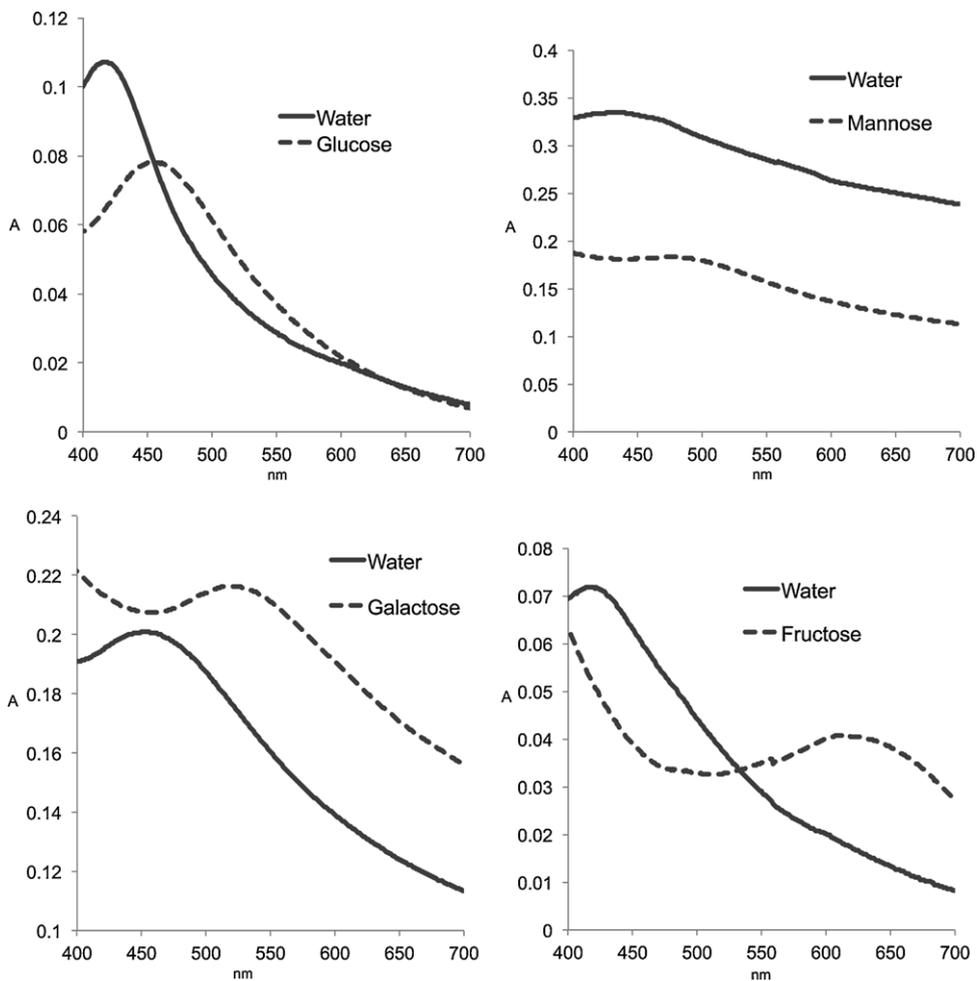


Fig. 5. An initially tuned-blue BCP film was exposed to four different sugar solutions containing either glucose, fructose, galactose or mannose at the same concentration. The difference of the peak wavelength observed in water versus that observed in the sugar solution was calculated to obtain the shift in wavelength. As seen in the figure, fructose gave the largest response of an approximately 200 nm redshift in wavelength. Galactose induced a 70 nm increase in wavelength and mannose and glucose induced smaller increases of approximately 40 nm each. This indicates that the BCP film can differentiate between fructose, galactose, mannose and glucose for a given concentration.

and redshift in color. Exposure to lower concentrations caused two boronic acid functionalities to bind to one glucose molecule. This effectively creates crosslinking in their hydrogel system, causing it to collapse and blueshift in color. Based on our results illustrated in Fig. 4 it is reasonable to assume that a similar phenomenon is occurring in the functionalized block copolymer system. The effect of pH on phenylboronic acid's ability to bind sugars such as glucose has been demonstrated. It has been shown that higher pH increases the K_{eq} of the glucose binding reaction while lower pH decreases the K_{eq} of binding glucose (Asher et al., 2003). Other photonic crystal chemical sensors have also shown successful response to a target analyte, particularly those fabricated from porous silicon (Lee and Fauchet, 2007; Li et al., 2003) or nanoprint lithography (Endo et al., 2010). The observed response in these systems, however, is small, non-visible, and thus cannot be measured without the aid of supplementary equipment.

3.4. Selectivity

The phenylboronic acid functionality introduced to the P2VP block of the BCP can covalently bind to any diol-containing sugar such as glucose, fructose, mannose and galactose. The K_{eq} of boronic acid binding to each one of these sugars varies depending on the sugar. This suggests that the boronic acid functionalized film should have a selective response to each simple sugar. To observe this effect, a BCP film functionalized and crosslinked to yield a blue color in water was exposed to 50 mg/ml solution of either glucose, fructose, mannose or galactose. After 30 min of exposure the BCP film's visible spectra was measured using UV–Vis spectroscopy. The peak difference in wavelength was then calculated as the difference between the peak wavelength of the film in water and in the sugar solution.

As seen in Fig. 5, the BCP film exposed to fructose gave the largest response of a 200 nm redshift in wavelength. The film exposed to galactose exhibited a 70 nm redshift in color. The smallest responses were from films exposed to glucose and mannose, which exhibited 44 nm and 37 nm decreases, respectively. Of note, in separate experiments the 50 mg/ml glucose solution evoked the same shift in wavelength (compare Fig. 4a to Fig. 5), although in each case the original color of the BCP film in water is different.

This difference in response between fructose, galactose and glucose is expected. It has been reported that fructose has the highest K_{eq} to bind to boronic acid, followed by galactose, and then glucose (Springsteen and Wang, 2002). This difference in binding is due to the steric structure of each sugar. Boronic acid can bind to both 1,2 and 1,3 cis diols but preferentially binds to 1,2 cis diols. Fructose for example has a planar 1,2 cis diol. Glucose does not have a 1,2 cis diol but has a 1,3 cis diol in its dominant pyranose form. Generally glucose binds to boronic acid in its furanose form, which does contain a planar 1,2 cis diol (Bielecki et al., 1999). This result signifies that for a given concentration, the boronic acid functionalized BCP film can differentiate between glucose, galactose and fructose.

4. Conclusions

Our results indicate that the PS-*b*-P2VP block copolymer film shows promise for detecting chemical targets. This colorimetric

material system is unique in that it requires only the functionalized block copolymer to indicate an exposure to a chemical target. Once functionalized with the 2-(bromomethyl)phenylboronic acid, the PS-*b*-P2VP film demonstrated the ability to respond to glucose with a change in color without the use of any supplementary enzymes such as glucose oxidase or additional equipment to assess the visible color change. The block copolymer lamellar stacks responded with a redshift in color in high concentrations of glucose and a blueshift in color for low concentrations of glucose. The BCP film also exhibited a selective response to fructose, glucose or galactose, by swelling to different degrees depending on which sugar is present. The PS-*b*-P2VP block copolymer can easily be processed into films, sheets or other large area coatings as needed. The color of the BCP film can be tuned to blue, green, yellow or orange as demonstrated. While similar sensors exist in porous silicon, lithography and hydrogel systems they lack the ease of fabrication and use of these block copolymer films and can require the use of a spectrometer to measure the shift in color. The results show the capabilities of photonic BCP films for chemical sensing as the functionalization can be exchanged for different sensing moieties.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.07.043.

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