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Boronic acid-based thin films that show saccharide-responsive multicolor changes

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ABSTRACT: A novel saccharide sensor that displays a distinct color change resembling a "traffic signal" was developed. By copolymerizing boronic acid and amine monomers on a glass plate, a boronic acid-containing thin film was obtained. Anionic blue and yellow dyes were adsorbed on the thin film, and the film was immersed in aqueous saccharide solutions containing a cationic red dye. With increase in the saccharide concentration in the solution, the thin film changes color from green to red via yellow. The observed distinct changes in color were attributed to a stepwise release and binding of dyes. The sensitivity of the saccharide sensor was dependent on the monomer composition of the thin film and increased with increasing the boronic acid content. The pH of the saccharide solution was another key factor affecting the sensing behavior, and glucose-responsive color changes were significantly enhanced at pH 7.8. By optimizing these conditions, significant color changes in response to glucose were achieved. Saccharide selectivity was found to be in the following order: fructose > glucose > galactose = mannose > sucrose. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42679.

KEYWORDS: dyes/pigments; films; molecular recognition; sensors and actuators

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INTRODUCTION

Saccharide sensors have attracted much attention because saccharides play a crucial role in the metabolic pathway of living organisms. To confront the medical challenges associated with increasing number of diabetic patients in both developed and developing countries,[1] it is essential to develop sensitive, inexpensive, and easily operable saccharide sensors for the personal diagnosis of diabetes. Most of the currently available saccharide sensors are based on enzymatic reactions. Although the enzyme-based systems have high selectivity, there are significant drawbacks, such as low durability and reproducibility because of unstable enzymatic activity.[2–4] Alternatively, boronic acid-based sensory systems have been extensively studied since the 1990s.[5–23] These studies have established that boronic acid is a powerful tool for the molecular recognition of saccharides in aqueous systems. However, boronic acid-based sensors sometimes do not display a distinct color change. Therefore, it is likely that introduction of a novel methodology for the development of practically applicable boronic acid-based sensors will be beneficial.

Our group has previously reported on the unique behavior of a saccharide-responsive polymer that was synthesized by the copolymerization of boronic acid monomer, amine monomer, acrylamide, and a crosslinker.[24] The protonated amino groups provide a positive charge to the polymer, which in turn allow for the adsorption of anionic dyes. When the polymer that has adsorbed both blue and yellow dyes is immersed in saccharide solutions, the two kinds of dyes are released in a stepwise manner: the blue dye is released first, and after a large portion of the blue dye has been released, yellow dye is released. Thus, the color of the solution clearly changes from colorless to green via blue. The proposed mechanism for the release of dye has been attributed to the formation of negatively charged boronic acid–saccharides complexes, by which originally adsorbed anionic dyes are replaced. Subsequently, we reported a short communication concerning the development of novel saccharide-sensing chips based on the above-mentioned saccharide-responsive polymer.[25] This sensing chip is prepared by polymerizing monomers on a glass plate, so that a thin film with saccharide responsiveness is formed. After anionic dye is adsorbed, the sensing chip is immersed in aqueous saccharide solutions. The thin film exhibits a saccharide-concentration-dependent color change resembling a "traffic signal", i.e., change in color from green to red via yellow.

Herein, we report on our detailed investigations into the multicolor saccharide-sensing chips. This study aimed to elucidate factors affecting on the responsiveness and to clarify response selectivity against various saccharides.

Additional Supporting Information may be found in the online version of this article.

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Table I. Monomer Compositions for the Preparation of Thin Films

	Monomer (mol dm^{-3})			
Sample	**4**	**5**	**6**	**7**
B0	0	0.10	0.85	0.10
B1	0.10	0.10	0.75	0.05
B2	0.20	0.10	0.65	0.05
B3	0.30	0.10	0.55	0.05

EXPERIMENTAL

Chemicals

Indigo carmine **1**, chrisophenine **2**, and propidium iodide **3** were purchased from Tokyo Chemical Industries (Tokyo, Japan). Boronic acid monomer **4** was synthesized according to the literature method.[26] Other reagents were purchased from Wako Pure Chemicals Industries (Osaka, Japan). All chemicals were reagent grade and used as received.

Synthesis of Thin Films

The saccharide-responsive thin film was prepared on a bare glass plate by a radical copolymerization of boronic acid monomer **4**, amine monomer **5**, acrylamide **6**, and methylenebisacrylamide **7**. Monomer compositions for the film synthesis are as shown in Table I. A mixture of dimethylsulfoxide : water = 9 : 1 was used for the preparations of monomer solutions, and potassium persulfate ($K_2S_2O_8$, 20 m*M*) was added as an initiator. To minimize interference in polymerization due to the presence of oxygen in air, all steps in the procedure were conducted under nitrogen atmosphere in a glove box. Each monomer solution (80 μL) was poured on an acrylic plate, and a sandwich was prepared by using a glass plate (48 × 28 mm) as the cover. The distance between glass and acrylic plates, i.e., the thickness of the monomer solution, was controlled by inserting a polyethylene film (45 μm thick) between the two. Polymerization was allowed to occur for 18 h at room temperature. Subsequently, the acrylic plate was removed, and the resulting thin film on the glass plate was washed with water. The obtained thin film was dried in air.

Staining of Thin Films

The thin film was stained by immersing the film on the glass plate in 10 m*M* HEPES buffer (50 mL; pH 7.4) containing different anionic dyes at room temperature for 12 h. The concentrations used to prepare the stained films were [**1**] = 60 μM and [**2**] = 20 μM.

Response to Saccharides

Stained thin films were independently immersed in different aqueous saccharide solutions (50 mL) at pH 7.0, 7.4, 7.8, and 8.2 (adjusted using 10 m*M* HEPES buffer) and 37°C in the presence and absence of cationic dye ([**3**] = 60 μM). The time-resolved color change in the thin film was recorded by monitoring the UV-vis absorption spectra of the film at normal incidence.

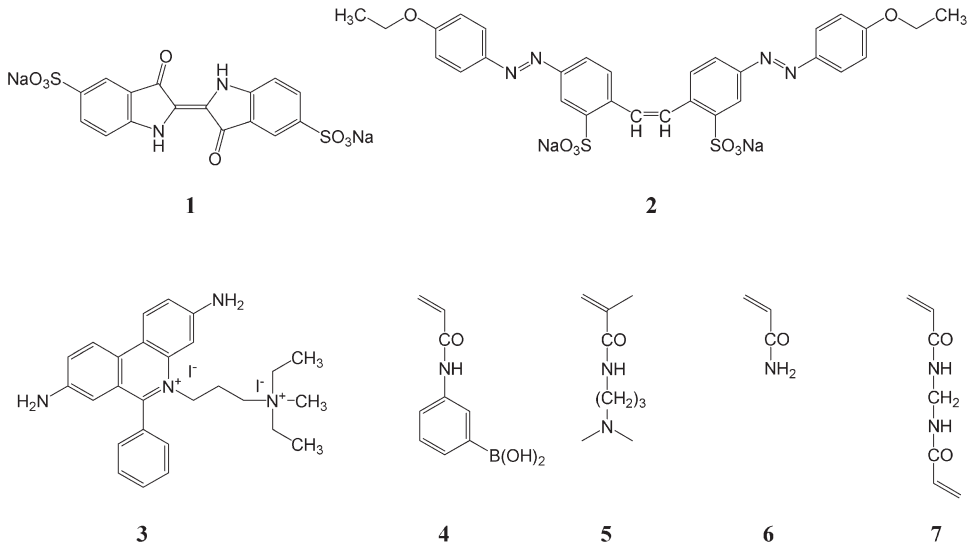
Apparatus

UV-vis absorption spectra were recorded using JASCO V-650 spectrometer. Scanning electron microscopy (SEM) images were obtained using JEOL JSM-6701F. Aqueous solutions were prepared with distilled water purified by Yamato WG202 system. pH values were measured using Metrohm 827 pH lab. ^{11}B NMR spectra were obtained on a JEOL JNM ECA-600 spectrometer.

RESULTS AND DISCUSSION

Preparation of Saccharide-Sensing Chips

For the preparation of a saccharide-responsive thin film, we polymerized a monomer mixture containing boronic acid monomer, amine monomer, acrylamide, and a crosslinker on a glass plate. The molecular structures are shown in Figure 1. The molar ratio between the boronic acid and the amine monomers is varied between 0 : 1 and 3 : 1, and each sample is named B0, B1, B2, and B3 (Table I). The resulting thin film is subsequently stained with anionic dyes (**1** and **2** in Figure 1). The film is stably adhered to the glass plate throughout the experiments. Because the surface of the glass is not modified prior to the polymerization, it is likely that the film adheres to the glass surface through noncovalent



1 **2** **3** **4** **5** **6** **7**

Figure 1. Molecular structures of the dyes and monomers.

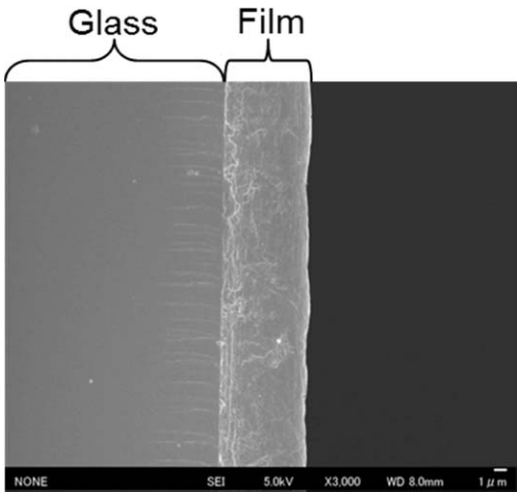


Figure 2. Cross-sectional SEM image of the thin film.

interactions, such as electrostatic interaction and hydrogen bonding with surface silanol groups. The film thickness of Sample B3 is ~7 μm, as determined by the SEM analysis of a cross-section (Figure 2).

Effect of Monomer Composition

To investigate the effect of monomer composition on the responsiveness toward saccharide, sensing chips with molar ratios between boronic acid and amine monomers of 3 : 1 (Sample B3), 2 : 1 (Sample B2), and 1 : 1 (Sample B1) are prepared. A sensing chip having higher boronic acid content (boronic acid : amine = 4 : 1) is prepared; however, the obtained thin film is fragile and readily peeled off from the glass surface. As a control sample, a thin film that does not contain boronic acid moiety (Sample B0) is prepared. After allowing for the adsorption of anionic dyes (**1** and **2**), these sensing chips are immersed in fructose solutions. The time courses of the color changes are shown in Supporting Information Figures S1–S3. The photographs of the samples immersed in various concentrations of fructose solutions for 2 h are shown in Figure 3. The color changes observed in samples B3 and B2 exhibit dependencies on fructose concentration; however, the change of Sample B2 is relatively weaker than that of Sample B3. In case of Sample B1, the color change is further diminished. It was confirmed that the boronic acid-free thin film (Sample B0) does not show fructose-responsive color change. The absorption spectra when the fructose concentration is 100 m*M* are compared in Figure 4. The intensity of the absorption peaks at 410 and 625 nm in case of Sample B0 remains unchanged before and after immersion in fructose solutions. Meanwhile, in case of Sample B1, an absorption peak is observed at 410 nm, whereas the peak at 625 nm is barely discernible. This implies that the blue dye is predominantly released from the thin film, whereas the yellow dye is retained. In case of B2, a new peak is observed at 525 nm in addition to the peak at 410 nm. This new peak indicates the binding of the red dye. Then, Sample B3 shows a strong absorption peak only at 525 nm, resulting in the appearance of red color in the thin film. These results clearly indicate that the boronic acid moiety is indispensable for the occurrence of the color change, and a higher boronic acid content leads to a higher responsiveness accompanying distinct color changes for the detection of saccharides.

To obtain direct evidence that the neutral boronic acid groups are converted to the negatively charged boronate groups upon binding with saccharides, we measured ^{11}B NMR spectra (Supporting Information Figure S11). In the absence of saccharides, 3-aminophenylboronic acid showed a single peak at around 9 ppm that is derived from the trigonal (neutral) boronic acid group. With increasing fructose concentration, a new peak assignable to the tetrahedral boronate group emerged at around 12 ppm.[27] In the presence of 100 m*M* fructose, it was found that almost all the boronic acid groups were converted to the negatively charged boronate groups.

According to the above-mentioned observations, the plausible mechanism for the multicolor changes is illustrated in Figure 5.

Effect of Ionic Strength

To investigate the effect of ionic strength on the color change, the thin film (Sample B3) was immersed in aqueous solutions containing various concentration of sodium chloride. As the results, it was found that the anionic dyes are only slightly desorbed even in the presence of 100 m*M* NaCl (Supporting Information Figure S12). Because the anionic dyes used are divalent, relatively stranger binding should occur between the thin film and the dyes through multiple electrostatic interactions. In addition, hydrophobic interaction may further strengthen the binding of the

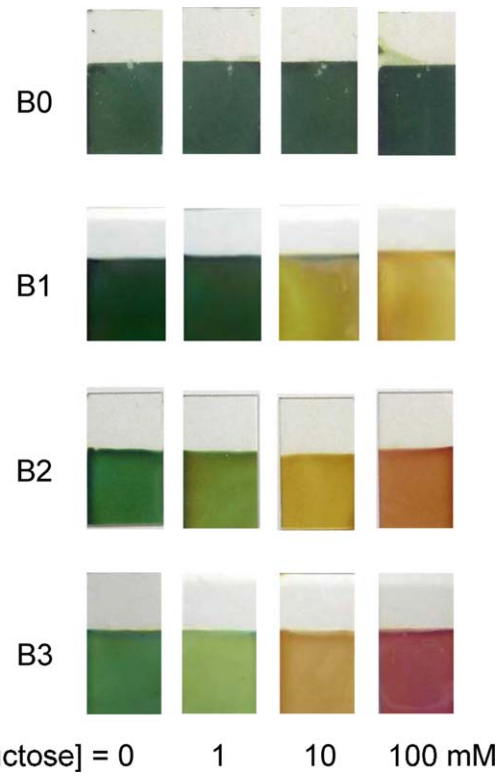


Figure 3. Effect of monomer composition on the response of the fabricated thin film for fructose. The thin films were immersed in aqueous fructose solutions containing **3** (60 μ*M*) at pH 7.4 for 2 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

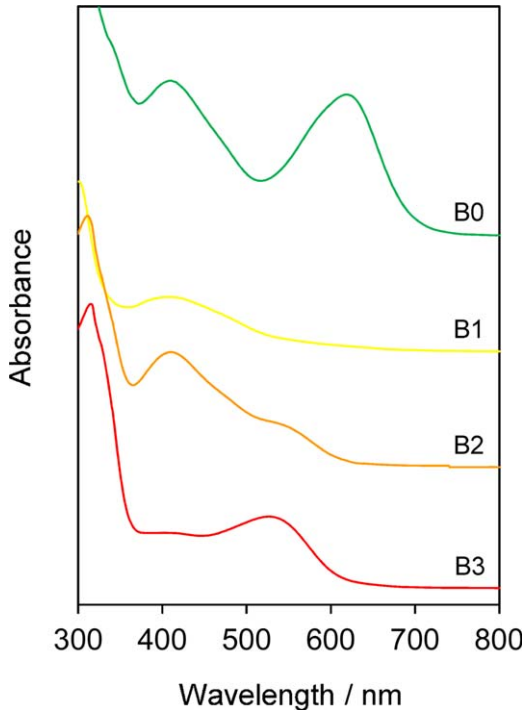


Figure 4. Absorption spectra of the thin films with varying monomer compositions. The measurement conditions are the same as those in Figure 3. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

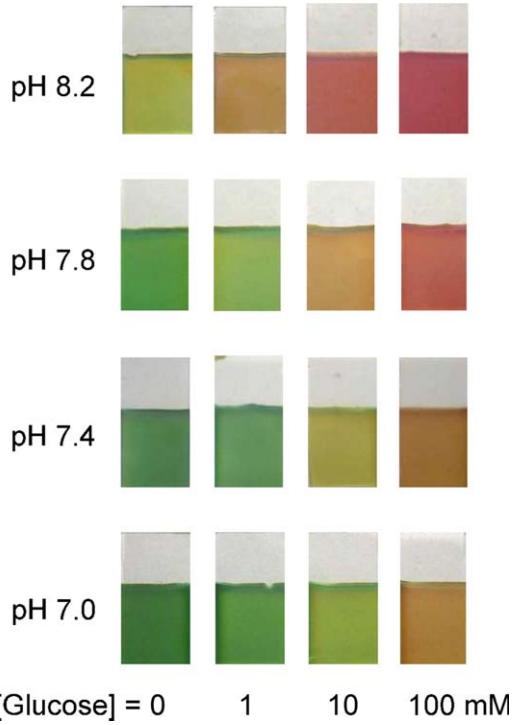


Figure 6. Effect of pH on the color changes of the thin films on immersion in aqueous glucose solutions containing **3** for 2 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

dyes to the thin films. Therefore, the present saccharide-responsive thin films are scarcely susceptible for the changes in ionic strength.

Response to Glucose

Because glucose is supposed to be the main target for medical diagnosis and process control in manufacturing industries, the response of the sensing chip (Sample B3) toward glucose is evaluated. The results are shown in Figure 6, and the time courses of the color changes are shown in Supporting Information Figures S4–S7. When the thin film is immersed in aqueous glucose solutions buffered at pH 7.4, the color change of the film is not as significant as that observed for the film in case of

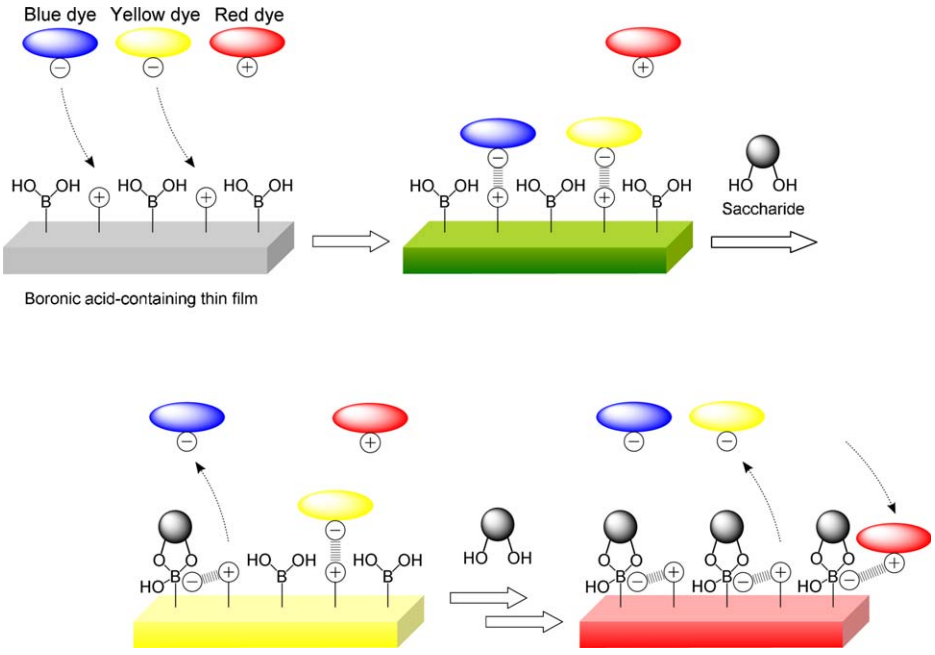


Figure 5. Plausible mechanism for the multicolor changes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

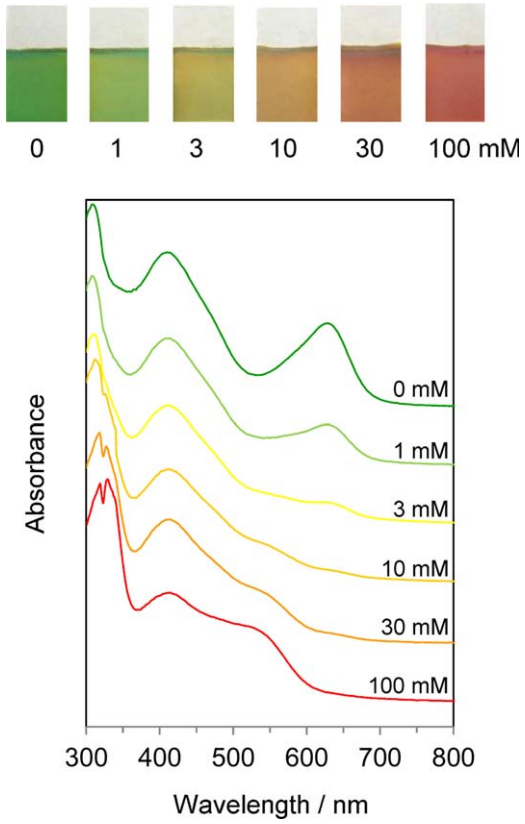


Figure 7. Changes in color and absorption spectra of the thin films after immersion in aqueous glucose solutions containing **3** (60 μM) at pH 7.8 (10 m*M* HEPES) for 2 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 8. Comparison of responsiveness against various saccharides. The measurement conditions are the same as those in Figure 3. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

fructose. The observed lower sensitivity against glucose is rationalized by the fact that the stability of the boronic acid–saccharide complex is much lower for glucose than it is for the complex with fructose.[28,29] Because alkaline conditions are known to favorably influence the complexation between boronic acid and saccharides,[30] the response of the film to the presence of glucose is evaluated at elevated pH. As seen from Figure 6, the response against glucose is enhanced at pH 7.8, and a distinct color change is clearly observed. When pH is elevated further to 8.2, a color change from green to greenish yellow occurs in the absence of glucose. With increasing pH, the dissociation of protons from the positively charged ammonium groups in the thin film should take place, and boronic acid groups should partly dissociate to negatively charged boronate groups. These phenomena result in diminished net positive-charge density in the thin film. Thus, the binding affinity of the film for the anionic dyes decreases, and the blue dye is replaced by the buffer molecules even in the absence of saccharides. We also studied the response of the film at lower pH. At pH 7.0, the response for glucose is relatively low when compared with that at pH 7.4. This can also be explained by the fact that the complexation between boronic acid and saccharide is pH dependent and suppressed under acidic conditions.

The above results indicate that the optimum condition for sensing glucose using the fabricated system is pH 7.8. Then, on examining detailed response profile for glucose at pH 7.8 (Figure 7), the thin film exhibits distinct color changes when the concentration of glucose is varied from 0 to 100 m*M*. A color change from green to yellowish green is noticeable when the concentration of glucose is 1 m*M*. At 3 m*M*, the film becomes yellow due to the nearly complete desorption of the blue dye. At higher concentration of glucose (10 m*M*), the film becomes pale orange, because the red dye is adsorbed under these conditions. Further increase in the glucose concentration to 30 and 100 m*M* leads to a gradual change in color to reddish.

Saccharide Selectivity

The results from the measurements to evaluate the selectivity of the thin film toward various saccharides are shown in Figure 8, and the time courses of the color changes are shown in Supporting Information Figures S8–S10. When the thin film is immersed in an aqueous 10 m*M* galactose solution buffered at pH 7.4, the film exhibits a slight color change to yellowish green. A more pronounced color change is observed when the concentration of galactose is 100 m*M*. Similar but slightly weaker changes in color are noticed in case of mannose. When the film is immersed in sucrose solutions, no noticeable change in color is observed throughout the concentration range. For a quantitative comparison of the changes in color observed for various saccharides, the ratio of absorbance peaks at 525 and 625 nm is plotted against the concentration of saccharides. It is clearly seen from Figure 9 that the sensor exhibits the highest sensitivity for

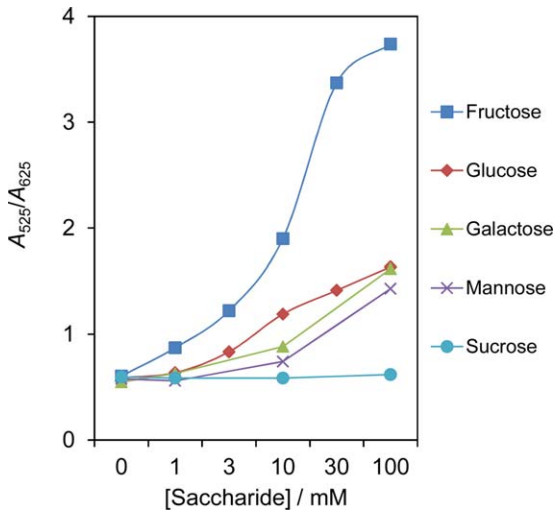


Figure 9. Plots of the ratio of absorbance peaks at 525 and 625 nm against the concentration of saccharide. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

fructose, and its response against glucose is marginally higher than that for either galactose or mannose. Virtually, no spectral change is observed in the case of sucrose. It is known that the binding affinity of boronic acids to saccharides decreases in the following order: fructose > galactose > mannose > glucose > sucrose.[28] Therefore, the observed high sensitivity for fructose can be attributed to the high binding affinity of boronic acid. However, the sensitivity for the detection of glucose seems to be higher than that anticipated from the stability order. This can be rationalized by the fact that a 1 : 2 complex is formed between glucose and boronic acid: glucose tends to form bis-boronate complexes, in which one glucose molecule is bound by two boronate groups, so that glucose shows much higher binding affinity for di-boronic acids than for mono-boronic acids.[31–33] Hence, the relatively higher sensitivity against glucose in this system seems to be arisen from the formation of bis-boronate complexes bound by two neighboring boronate groups in the thin film.

CONCLUSIONS

We have developed a novel saccharide-sensing chip that displays its response by distinct color changes in the pattern of a "traffic signal." By copolymerizing a boronic acid monomer and an amine monomer on a glass plate, we obtained a boronic acid-containing thin film. After adsorbing anionic blue and yellow dyes, the film was immersed in aqueous saccharide solutions. A saccharide concentration-dependent change in the color of the film from green to red via yellow was demonstrated.

The sensitivity of the thin film toward saccharides was dependent on the monomer composition of the thin film. In the absence of the boronic acid unit, the film showed virtually no color change. The sensitivity increased with the boronic acid content in the film. The pH of the saccharide solution was found to be another key factor affecting the sensing behavior. At pH 7.4, response of the film for glucose was not as sensitive as that for fructose, whereas at pH 7.8, glucose-responsive color changes were significantly enhanced. We also examined saccharide selectivity and found that the sensitivity was in the following order: fructose > glucose > galactose = mannose > sucrose.

The present system is particularly advantageous because (1) preparation is relatively simple and does not require complex organic synthesis and (2) any anionic and cationic dyes can be utilized, offering a variety of color changes. We believe that this methodology can be a basis for developing practically applicable saccharide sensors in the near future.

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