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Potentiometric Saccharide Detection Based on the pK_a Changes of Poly(aniline boronic acid)

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Abstract: A novel approach for the potentiometric detection of saccharides using poly(aniline boronic acid) is presented. A model is described in which the electrochemical potential is sensitive to the change in the pK_a of the conducting polymer as a result of boronic acid−diol complexation. In this system, boronic acid complexation is the mode of transduction and it is manifested as changes in the electrochemical potential of the polymer with remarkable selectivity. Characteristics of both transient and steady-state response associated with the complexation are discussed. The presence of Nafion and fluoride during the electrochemical polymerization of 3-aminophenylboronic acid are shown to impact the sensitivity and the stability of the electrode response. The sensor sensitivity is improved significantly by increasing the concentration of sodium fluoride during the polymerization. Finally, the nature of the selectivity of the boronic acid−diol reaction under these conditions is explored by using molecular orbital calculations.

Introduction

One of the most successful and historically important electrochemical approaches for detecting saccharides uses enzyme-based electrodes. These have been under development since the 1960s.[2] To date, the *in situ* detection of saccharides, including D-glucose and D-fructose, has been the focus of considerable research due to the importance of saccharide monitoring in such diverse areas as medical diagnostics[3,4] and bioprocessing.[5] The use of enzyme-based monitoring approaches in these areas has been hampered due to the fact that enzyme-based sensors typically require mediators, their steady-state response is a function of the mass transport of the analyte, and they consume the analytes of interest. In systems using oxidoreductases, oxygen/peroxide is widely used as the mediator;[4] however, in this configuration a large excess of oxygen is required for the limiting current to be proportional to the concentration of the analyte, and fluctuations in oxygen concentrations can result in variations in sensitivity. In recent years these issues have been addressed through the use of mediators other than oxygen[6−8] and through the direct oxidation of the enzyme.[9−11] However, many difficulties remain[4] and there is considerable interest in developing alternative sensing approaches for the continuous monitoring of saccharides.

Sensors based on the complexation of boron compounds with saccharides are an attractive alternative to enzymatic approaches. The first reports of the complexation of saccharides with boric[12,13] and boronic[13] acids appeared in the 1950s, and this pioneering work has since attracted considerable attention. To date, boronic acid-based detection schemes have proven important for saccharide detection.[14−18] Boronic acid chemistry has been employed in a number of sensing strategies including direct pH measurements,[19] fluorescence,[20−22] UV−vis,[23,24] circular dichroism,[14,25] near-IR,[26] surface plasmon resonance

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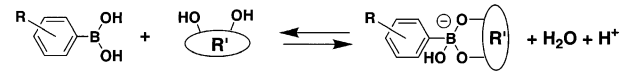
Scheme 1



spectroscopy,[27] potentiometry,[28−30] conductance measurements,[12,27,31] and quartz crystal microbalance measurements.[27] These approaches overcome many of the issues that plague enzymatic systems as described above. Most importantly, these approaches are "reagentless" and their sensitivity is not dependent on the mass transport of analyte.

The complexation of saccharides (as well as alkyl and aromatic diols) with aromatic boronic acids produces a stable boronate anion[12] and a proton (1:1 stoichiometry) in the pH range 6−10 (see Scheme 1). Since the complexation is reversible,[32,33] saccharides bind to aromatic boronic acids as a function of the saccharide concentration in the physiological pH range 6.8−7.5. The percentage of complex produced with phenylboronic acid at pH 7.4 is ca. 30% for D-glucose and ca. 80% for D-fructose (boronic acid:saccharides = 1:2 (molar ratio)).[33] While the degree of complexation is significant at high molar concentrations, for analytical applications in which the saccharide concentration is relatively small, increasing the percentage of boronate complex near neutral pH remains an important goal. Since complexation involves a tetrahedral boronate anion as an intermediate,[34] binding constants are increased at higher pH. Two approaches have been used successfully to increase binding constants of phenylboronic acid with saccharides at neutral pH: (1) the addition of neighboring amine groups[35] or fluoride[36] to form a stable covalent bond with the boron atom, thereby driving the boron from sp^2 to sp^3 hybridization, and (2) the addition of an electron-withdrawing group to the phenyl ring to shift the pK_a to a lower value.[33] The latter approach illustrates the interdependence of the complexation reaction and the electronic structure of the adjoining aromatic system. This relationship is further demonstrate by recent work where complexation involving boronic acid-substituted ferrocene resulted in a corresponding change in redox potential.[29,37] This shift in potential can be understood in terms of the formation of the boronate anion, which can result in a change in the inductive[38] and resonance properties of the boron-containing substituent.[39,40]

Given the above knowledge that complexation and the nature of the aromatic system are interdependent, we have undertaken a study of the complexation reactions occurring when the aromatic system is part of a conducting polymer. The oxidation state of a conducting polymer is readily varied and hence may be used to tune the electronic properties of the polymer's backbone, thereby influencing the properties of the boronic acid moiety and hence its complexation with saccharides. In addition, since the complexation changes the electronic properties of the boronic acid substituent, we can monitor the binding event by measuring changes in the electrochemical potential of the polymer.

Conducting polymers[41] have indeed attracted considerable attention for use in sensor applications.[42] Polyaniline in particular has been the focus of considerable research interest due to its stability, high conductivity, ease of production, and pH dependent behavior,[43−45] as well as its mediation/catalytic abilities[46−48] Although there have been numerous accounts in the literature over the years on the preparation of functionalized polyanilines, only recently has boronic acid-substituted polyaniline been reported.[49,50] In these reports, it has been shown that aminophenylboronic acid can be polymerized as a copolymer with aniline or as a homopolymer through a fluoride-catalyzed reaction.[49,50] In the latter case, it was shown that complexation of fluoride with the boronic acid moiety substantially reduced the oxidation potential required for polymerization, thereby eliminating deleterious side reactions that occur at more positive potentials. This breakthrough has opened up the possibility of developing new electrochemical approaches to saccharide detection using poly(aniline boronic acid) (PABA).[30]

In this work we provide a detailed investigation into the nature of the boronic acid−saccharide complexation in PABA and its influence on the electrochemical potential of the polymer. Specifically, we explore the effect of the changing inductive and resonance effects that occur upon complexation on the electrochemical potential. Our findings demonstrate that in addition to transient fluctuations associated with short-lived pH changes, complexation also results in a steady-state change in the electrochemical potential that is dominated by a change in the pK_a of the polymer. Furthermore, we explore the nature of the selectivity of the response in terms of the distribution of saccharide isomers and the preferential complexation between boronic acids and *cis*-cyclopentanediols. Molecular orbital calculations are used to reveal the important parameters that predict sensitivity in this system. Finally, the role of Nafion and fluoride used in the polymerization on the sensitivity and stability are explored.

Experimental Section

Materials. 3-Aminophenylboronic acid hydrochloric salt, D-glucose, D-fructose, α-methyl-D-glucoside, *cis*-1,2-cyclopentanediol, *trans*-1,2-cyclopentanediol, *cis*-1,2-cyclohexanediol, *trans*-1,2-cyclohexanediol,

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and Nafion solution (perfluorinated ion-exchange resin, 5 wt % lower aliphatic alcohol and 45% water) were purchased from Aldrich Chemical Inc. Bulk distilled water was first filtered and ion exchanged to yield 18.3 MΩ quality water using an EasyPure RF, Barnstead Thermolyne model 7031. Phosphate buffer saline (PBS) stock solution (pH 7.4) was purchased from EM Science. Unless otherwise noted, purchased chemicals were used as received.

Electrochemical Measurements. Cyclic voltammetry (CV) was performed at 25 °C using a EG&G 362 potentiostat with an EG&G 175 universal programmer and a Hewlett-Packard 7046B *X*−*Y* recorder. Measurements were taken in a three-electrode cell configuration by using a glassy carbon disk (BAS, 3.0-mm diameter) as the working electrode, a Pt-coil auxiliary electrode, and a Ag/AgCl reference electrode. Unless otherwise noted, all CV experiments were conducted in a 0.5 M HCl aqueous solution and at a scan rate of 100 mV s^{-1}.

Electrochemical Production of PABA. PABA was produced electrochemically on a glassy carbon electrode.[50] The reaction procedure and conditions were as follows: 3-aminophenylboronic acid hydrochloride salt (87 mg, 40 mM) and sodium fluoride (21 mg, 40 mM) were dissolved in 0.2 M HCl solution 12.5 mL. Nafion solution (2 mL) was added and the solution mixture was vigorously stirred. The potential was scanned between 0.0 and +1.1 V in the unstirred solution until the charge in the cathodic scan reached 10 mC cm^{-2}. The deep bluish-green film that was obtained was washed with water and placed in a 0.1 M HCl solution to verify its redox behavior with CV. The potential window for CV measurement was 0.0−0.8 V. After verifying that the redox response was stable, the potential was scanned to +0.8 V and held at that potential for 10 s. The electrode was then rinsed with water, followed with PBS solution, and then soaked in PBS solution for 24 h to allow the electrochemical potential to stabilize. At this point, the polymer film was a deep blue color. PABA produced in the presence of higher sodium fluoride concentrations (105 mg, 200 mM) was prepared in an identical fashion with the exception of the absence of Nafion and the use of 0.5 M HCl.

Open-Circuit Measurements. Open-circuit potential measurements (E_{oc}) were performed by using a CH Instrument, CHI-660 workstation controlled by PC. Measurements were taken in a three-electrode cell configuration with a polymer-modified (PABA) glassy carbon disk (BAS, 3.0-mm diameter) as the working electrode, a Pt-coil (0.5-mm diameter, 50-cm length) auxiliary electrode, and a Ag/AgCl reference electrode. Unless otherwise noted, all open circuit experiments were conducted in a stirred PBS (pH 7.4) solution. The electrode potential was allowed to settle in PBS solution prior to performing all open-circuit measurements.

Flow Cell and Pump Controlled Experiments. To examine the performance of a PABA electrode under dynamic concentrations, an HPLC quat pump (Hewlett-Packard, G1311A) with degasser (Hewlett-Packard, G1322A) was used with a specially designed flow cell fabricated in house. PABA was produced on the glassy carbon electrode at the higher NaF concentration, as described above. Electrical isolation between the pump and flow cell was required to reduce noise and to obtain a stable and reliable response. To achieve this, two isolation points were placed in the setup. Separate bottles containing PBS and 100 mM of D-fructose in PBS were connected, and the concentration of the analyte was programmed to achieve 0, 5, 0, 10, 0, 20, 0, 10, 0, 5, 0 mM at a delivery rate of 1 mL min^{-1}. Each concentration was held constant for 10 min and changes in concentrations were achieved within a 30-s time period.

Computational Calculations. Geometry optimization for each possible quasi-stable structure was performed using MOPAC (AM1 and PM3) and MM2 in Chem3D software version 4.0 (CambridgeSoft Corp.). RMS: 0.001 was set for the calculation of MM2, AM1, and PM3 with all standard parameters.

Responses of Analytes. After the PABA electrode was allowed to settle, the change in E_{oc} was monitored before and after the injection of an aliquot of analyte stock solution by using a microsyringe. The electrode was used repeatedly (at least 5 times) before being rinsed with water and equilibrated in PBS. No problems with adhesion were observed.

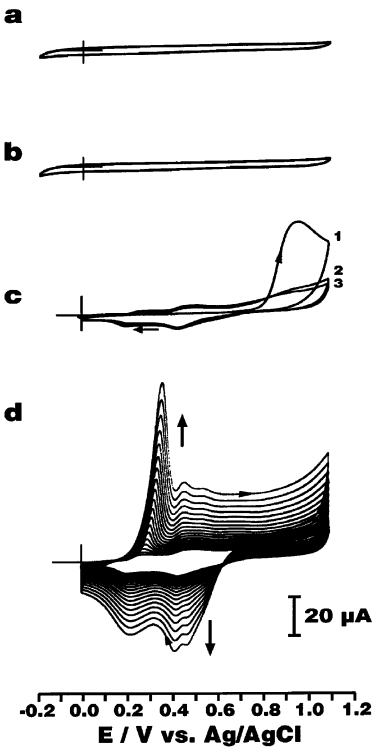

Figure 1. Cyclic voltammogram of a GC electrode in (a) 0.5 M HCl blank, (b) 40 mM phenylboronic acid plus 200 mM NaF in 0.5 M HCl, and (c) 40 mM aminophenylboronic acid plus 200 mM NaF in 0.5 M HCl and (d) subsequent scans of (c). Scan rate: 100 mV s^{-1}.

Results and Discussion

Production of PABA. The impact of fluoride complexation with aromatic boronic acids is illustrated by the significant increase in the solubility of phenylboroninc acid in both neutral and acidic solutions upon the addition of fluoride. The increase in solubility is a result of the formation of the fluorinated boronate anion.[51] Figure 1a and b demonstrate that the fluorinated boronate anion species is electrochemically inert within the potential window between −0.2 and +1.1 V. In contrast, 3-aminophenylboronic acid is readily oxidized in the presence of fluoride at approximately +1.0 V (see Figure 1c). Continued cycling of the potential results in the growth of PABA on the electrode surface in agreement with previous reports.[50]

It has been found that the stability and pH range of polyaniline films can be enhanced by the addition of polyanioninc materials such as Nafion.[52] The electrochemical polymerization of 3-aminophenylboronic acid was achieved both in a Nafion suspension and at a Nafion-coated electrode, and there were no significant differences in the polymerization behavior or the redox characteristics of the films.

Open-Circuit Potential Measurements. Polyaniline **1** (see Scheme 2) consists of benzenoid diamine and quinone diimine groups.[53−55] The distribution of these groups is a function of

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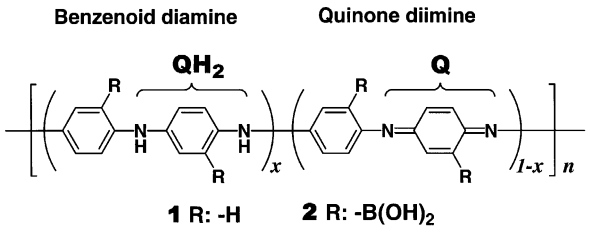
Scheme 2



the oxidation state of the polymer and the degree of protonation of the polymer is a function of the pH (the pK_a of protonated amine and imine are 2.5 and 5.5, respectively).[56,57] Since the redox chemistry of **1** involves both electron and proton-transfer processes, the electrochemical potential is sensitive to changes in pH. More precisely, the dependence of the electrochemical potential on pH can be described by a double-square scheme.[58] Under conditions where the system appears to undergo a thermodynamically reversible $2e^-2H^+$ redox reaction, the Nernstian expression simplifies to

$$E = E^{\circ\prime}_{Q/QH_2} + (RT/2F)\ln[Q][H^+]^2/[QH_2] \quad (1)$$

Alternatively, it can be shown that the electrochemical potential will be a function of the K_a of the protonated quinone diimine QH_2^{2+}. Assuming a concerted $2e^-$ $2H^+$ reaction, the acid−base reaction associated with the quinone diimine group in eq 1 can be written as

$$Q + 2H^+ \rightleftarrows QH_2^{2+} \quad (2)$$

with the corresponding acid dissociation constant:

$$K_a = [Q][H^+]^2/[QH_2^{2+}] \quad (3)$$

The reduction of the protonated quinone diimine structure is given by

$$QH_2^{2+} + 2e^- \rightleftarrows QH_2 \quad (4)$$

with the corresponding Nernst expression:

$$E = E^{\circ\prime}_{QH_2^{2+}/QH_2} + (RT/2F)\ln[QH_2^{2+}]/[QH_2] \quad (5)$$

The net proton coupled redox reaction (combining reactions 2 and 4) is then

$$Q + 2H^+ + 2e^- \rightleftarrows QH_2 \quad (6)$$

Substituting the expression for $[QH_2^{2+}]$ from eq 3 into eq 5 yields the corresponding Nernst expression for the net reaction, which is a function of proton concentration as well as K_a:

$$E = E^{\circ\prime}_{QH_2^{2+}/QH_2} + (RT/2F)\ln[Q][H^+]^2/K_a[QH_2] \quad (7)$$

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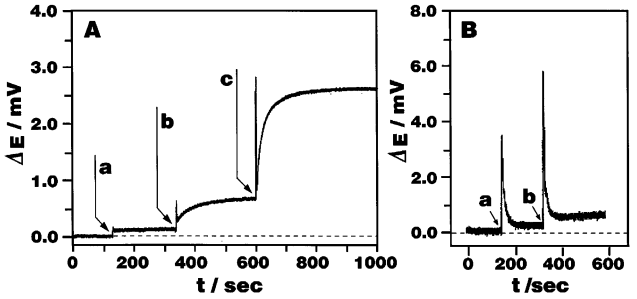

Figure 2. (A) Response curve of a poly(anilineboronic acid) electrode as a function of time upon addition of 6.8 mM: (a) α-Methyl-D-glucoside; (b) D-glucose; (c) D-fructose in pH 7.4 PBS. (B) Response of poly-(vinylphenylboronic acid) coated polyaniline upon two additions of 3.4 mM D-fructose in pH 7.4 PBS.

Changes in the properties of a substituent group will therefore influence the electrochemical potential of a proton coupled redox reaction in two ways: (1) altering the formal potential of the redox couple $E^{\circ\prime}_{QH_2^{2+}/QH_2}$ and (2) altering the acid dissociation constant, K_a.[59] In the case of the conversion of the boronic acid to the boronate complex by cis diol (Scheme 1), it is expected that there will be changes in both the inductive and resonance properties of the boron moiety.[39] Specifically, formation of the boronate complex leads to an increase in its inductive electron-donating ability, while eliminating the mesometrically electron-withdrawing nature associated with the vacant p-orbital on the boron.[40] Under conditions where dE/dpH = 0 (e.g., both oxidized and reduced forms are completely dissociated), an increase in electron-donating ability of a substituent will decrease the formal potential. This behavior has in fact been observed for ferrocene boronic acid, where a −140 mV change in formal potential is reported between the uncomplexed and complexed species.[29] On the other hand, increasing the electron-donating ability of a substituent is expected to stabilize the acid form (i.e., QH_2^{2+}). As such, converting boronic acid into the boronate anion complex is also expected to reduce the formal potential as well as to reduce the K_a of the protonated quinone diimine group. It becomes apparent that the two processes offset one another as indicated in eq 7, although the net effect (discussed below) will depend on the relative magnitude of the two influences and is ultimately expected to be a function of the binding constant and concentration of the analyte.

The influence of both the transient pH change and the net thermodynamic effect of complexation on E_{oc} are shown in Figure 2 where saccharides with varying binding constants (D-fructose > D-glucose > α-methyl-D-glucoside)[60] are added. The E_{oc} spikes generated upon addition of a saccharide are attributed to transient pH changes that occur upon complexation (see Scheme 1 and eq 7). The rapid decrease in the E_{oc} is likely due to diffusion of protons from the film into the bulk solution. In fact, similar transient profiles are observed (see Figure 2b) using a composite electrode consisting of poly(vinylphenylboroinic acid)-coated polyaniline/GC electrode, where the polyaniline-coated electrode acts as a pH-sensitive electrode.

The gradual change in E_{oc} observed in Figure 2a is due to the net effect of complexation on the electrochemical potential. The increase in potential suggests that K_a change plays a greater

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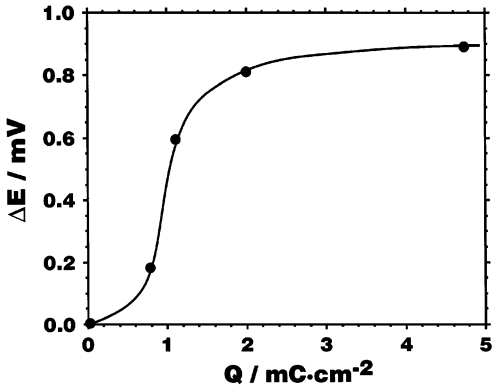

Figure 3. Sensitivity of open circuit response for 10 mM addition of D-glucose as a function of film thickness as indicated by the total charge measured during the reduction of the deposited film. Polymerization conditions: 40 mM 3-aminophenylboronic acid, 40 mM NaF electropolymerized through a Nafion thin film (2 μL of Nafion alcohol solution was cast onto a 3-mm diameter glassy carbon electrode and dried under room temperature for 2 h).

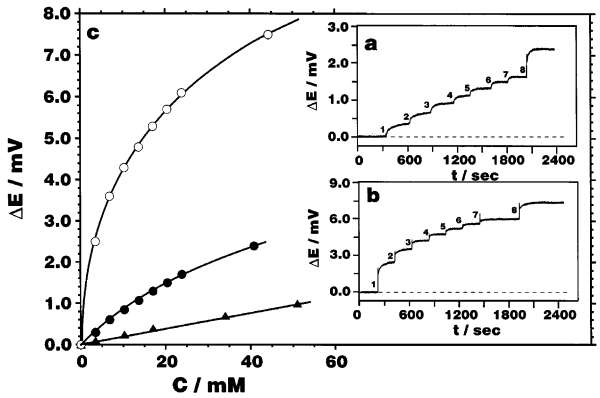

Figure 4. Saccharide response curve of a PABA electrode in pH 7.4 PBS as a function of time. Additions resulted in the following series of concentrations: (1) 3.4; (2) 6.8; (3) 10.2; (4) 13.6; (5) 17.0; (6) 20.4; (7) 23.8; (8) 40.8 mM. (a) D-Glucose and (b) D-fructose response curves of a poly(anilineboronic acid) electrode in pH 7.4 PBS as a function of time. (c) Calibration curves for D-glucose (●) and D-fructose (○) using a PABA electrode and of D-glucose (▲) for polyaniline coated electrode in pH 7.4 PBS.

role than the change in the formal potential under these conditions. This is likely due to the increase in electron density ortho to the boronate complex, directly impacting the K_a of the protonated amine.[39] Furthermore, Figure 2a shows that the equilibrated E_{oc} is a function of the binding constant of the saccharide.

Sensor Characteristics. Sensor stability and sensitivity were influenced by both the presence of Nafion and the polymerization conditions. Initial polymerization conditions made use of a 1:1 concentration of fluoride to monomer as reported previously.[50] It was found that incorporation of Nafion under these conditions improves stability of the films, presumably due to electrostatic interaction between PABA and Nafion. Delamination of the films was observed in the absence of Nafion during stirring of the solution, while the films grown in the presence of Nafion adhered well. Composite sensors made in this fashion demonstrated good selectivity and sensitivity consistent with reported binding constants (i.e., D-fructose > D-glucose). Sensitivity increased with the amount of polymer deposited over a limited range (see Figure 3) and increased to a limiting value, suggesting that the transition is likely associated with complete coverage of the electrode surface.

The selectivity of the boronic acid complexation for various saccharides is influenced in part by their isomeric composition in aqueous solution.[61] Specifically, when a saccharide is dissolved in water, mutarotation, which can occur on a time scale of minutes, leads to an equilibrium mixture of four forms: α-pyranose, β-pyranose, α-furanose, and β-furanose. In the case of D-glucose the distribution at equilibrium under neutral conditions is 39.4, 60.2, 0.2, and 0.21%, respectively,[62] whereas the distribution for D-fructose is 2.0, 68, 6.0, and 23%, respectively.[63] ^{13}C NMR studies have revealed a five-membered ring containing 1,2-*cis*-hydroxyls is involved in the complexation of D-glucose and D-fructose, corresponding to the β-furanose form of D-fructose (23%)[63,64] and the α-furanose form of D-glucose (2%).[65] These findings suggest that complexation occurs preferentially with the *cis*-hydroxyls and the anomeric hydroxyl under neutral conditions, and that the geometry of the five-membered ring is critical. Thus, it can be concluded that the apparent sensitivity of a boronic acid-based sensor will also be a function of the binding constants of the various isomers and their relative distributions in solution (*vide infra*).

Figure 4 shows the calibration curves for D-fructose and D-glucose. Although the selectivity and sensitivity of the boronic acid complexation is clearly retained in the electrode response, sensitivity is modest (ca. 1−4 mV mM^{-1}). To investigate the effect of fluoride on the polymerization and sensitivity of the resulting electrodes, the concentration of sodium fluoride was increased from 40 to 200 mM.[66] Since the complexation of fluoride with boronic acid leads to a negatively charged species,[67] PABA should have a self-doped structure.[50] Further, since the complexation shifts the formal potential of an outer-sphere redox couple (ca. 59 mV pF^{-1}) over a large concentration range (from 1 mM to 5 M),[17] fluoride concentration should have a significant effect on the polymerization rate and in turn the morphology[68] and behavior of the polymer.[69] Indeed, increasing the concentration of sodium fluoride resulted in significant negative shifts in the oxidation potential, thereby enhancing polymerization rates, and is consistent with the formation of a negatively charged substituent[17] and an increase in pH due to the formation of HF (pK_a = 3.1).

In addition to enhancing the electropolymerization process, higher fluoride concentrations significantly increased the sensitivity. As shown in Figure 5a, a 3-fold enhancement in sensitivity is seen compared to the addition of the same quantity (10 mM) of analyte to a polymer grown under low fluoride conditions (e.g., Figure 4, addition 3). Reversibility of the complexation (see Figure 5b) is also maintained as indicated by a return to baseline. The excellent selectivity of electrodes grown in the presence of higher sodium fluoride concentrations

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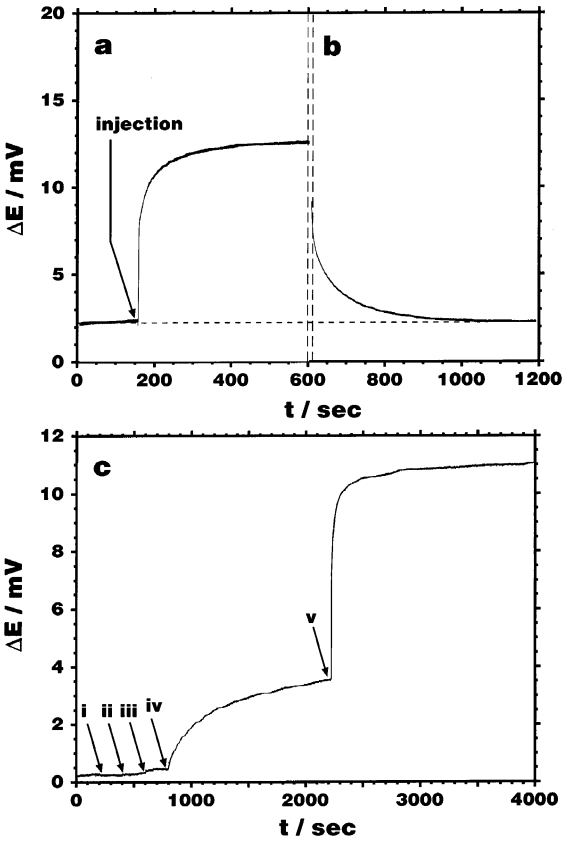

Figure 5. PABA (film was made from 40 mM 3-aminophenylboronic acid, 200 mM NaF), (a) 10 mM D-fructose injection, (b) soaked in the PBS solution (no fructose added). (c) Open-circuit potential measurement for (i) ethyl alcohol, (ii) ethyleneglycol, (iii) α-methyl-D-glucoside, (iv) D-glucose, and (v) D-fructose. Each concentration was 3.4 mM.

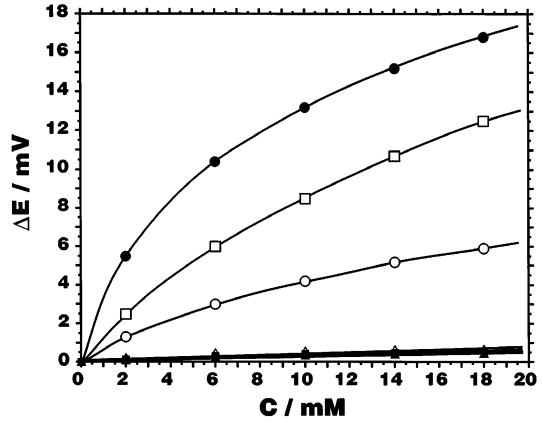

Figure 6. Sensitivity of PABA to D-fructose (●), D-glucose (○), *cis*-1,2-cyclopentanediol (□), *trans*-1,2-cyclopentanediol (△), *cis*-1,2-cyclohexandiol (■), and *trans*-1,2-cyclohexanediol (▲) and in PBS.

is shown in both Figure 5B and Figure 6. Figure 5B demonstrates that the sensor is relatively insensitive to linear alcohols and 1,2-alkanediols. Similarly, Figure 6 shows that the sensor response is insensitive to cyclic 1,2-alkanediols with the exception of *cis*-1,2-cyclopentanediol.

The time dependence of the electrochemical potential upon the addition of complexing diols did not follow a simple model for non-steady-state diffusion into a thin film on top of an impermeable substrate.[70] This behavior is likely due to complications associated with diffusion coupled with complexation as well as structural changes in the polymer. However, it was possible to estimate an effective diffusion coefficient from the initial rate of sorption[70] by using

$$\frac{M_t}{M_\infty} = \frac{4}{\pi^{1/2}}\left(\frac{Dt}{l^2}\right)^{1/2} \tag{8}$$

where M_t is mass uptake as a function of time t, D is the diffusion coefficient, and l is the film thickness. Assuming that the measured electrochemical potential is proportional to mass uptake and determining the slope of the linear portion of a plot of M_t/M_∞ versus $t^{1/2}$ ($M_t/M_\infty < 0.5$) and a film thickness of approximately 0.5 μm (the film thickness was estimated on the basis of total charge passed during polymerization[71]), an effective diffusion coefficient of 2×10^{-12} cm^2 s^{-1} was estimated for D-fructose. This value is about 2 orders of magnitude less than that reported for methanol diffusion through polyaniline,[72] which is consistent with both the larger molecular size of fructose and the coupled complexation process. These results suggest that the response is largely diffusion controlled and that any factor that accelerates mass transport through the film will shorten the response time.

It is interesting to note that the time required to reach steady state is noticeably longer for D-glucose than for D-fructose (e.g., Figure 5B). This difference is likely due to a displacement of the equilibrium among the different isomeric forms of D-glucose.[61] Complexation with boric acid is known to displace the relative concentrations of various isomers of D-glucose *via* mutarotation.[33,73,74] Since the percentage of the complexing form of D-glucose (α-furanose, 0.2%) is small relative to that of D-fructose (β-furanose, 23%) and since the rate of mutarotation of D-glucose is significantly slower than that of D-fructose, it is expected that tautomerization-induced response changes that occur slowly as a function of time will be more significant in the case of D-glucose relative to D-fructose.

In addition to increased sensitivity, polymer films prepared with higher concentrations of fluoride exhibited better stability and adhesion properties. Removing Nafion from the system had the effect of eliminating the potential spikes associated with transient pH changes observed in Figure 2. This behavior is likely due to the permselective nature of Nafion, in which the negatively charged sulfonate groups attached to the polymer backbone repel solution phase anions.[75] By excluding phosphate anions, the buffer capacity inside the film will be reduced,[76] making the local pH changes more dramatic. This hypothesis is supported by the return of potential spikes (similar to those seen in Figure 2) upon dilution of the PBS solution by an order of magnitude, thereby reducing its buffering capacity.

Selectivity Based on Boronic Acid−Diol Interactions. To determine the structural parameters that determine the sensitivity

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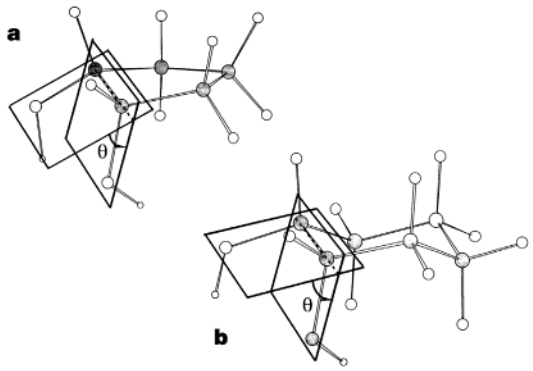


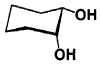
Figure 7. Dihedral angle for two hydroxy groups of *cis*-1,2-cyclopentanediol and *cis*-1,2-cyclohexanediol.

of our approach, we performed molecular orbital calculations on cyclic alkanediols. Phenylboronic acid is known to form stable complexes with *cis*-1,2-cyclopentanediols but not with *trans*-cyclopentanediols. However, complexation with 1,2-cyclohexane is fairly weak for both *cis*- and *trans*-cyclohexanediols.[12] As seen in Figure 6, the results obtained with our system are in good agreement of these observations, i.e., that there is a dramatic difference in sensitivity between *cis*- and *trans*-1,2-cyclopentanediol and virtually no response for *cis*- and *trans*-cyclohexanediol. This behavior has been attributed to the steric constraints imposed by the different structures and this assertion has been used to explain the remarkable selectivity of this complexation for other diols and sugars.[12,73]

More detailed studies have shown that the selectivity of 1,2-diols decreases in the order five-membered-*cis* > six-membered-*cis* > six-membered-*trans* isomers.[60] This study revealed several interesting points in addition to the *cis*/*trans* stereoselectivity of five- or six-membered rings. For example, there is a remarkable ring-size selectivity of *cis*-diols, as indicated by the ratio of binding constants (ca. 200) for *cis*-cyclopentandiol/*cis*-cyclohexanediol.[60] The affinities of cyclic 1,2-diols are correlated with the smaller dihedral angles between the adjacent C−OH groups, and earlier work using molecular mechanic calculation (MM2) by Oshima *et al.*[60] suggests that the five-membered cis isomer has a more planar conformation, thereby facilitating the complexation with boronic acid groups. To explore our experimental results that indicate that *cis*-1,2-cyclopentanediol has a more favorable complexation in comparison to other diols, AM1 and PM3 (MOPAC) molecular orbital as well as MM2 molecular mechanics calculations were performed to estimate the dihedral angles in addition to the calculation of oxygen−oxygen interatomic distances (see Figure 7).

As seen in Table 1, there is no significant difference between the results obtained by either computational method. However, for *cis*-1,2-cyclopentanediol, the calculated dihedral angles have a strong dependence on the Hamiltonians used, and all calculated angles for *cis*-1,2-cyclopentanediol were significantly smaller than for the other three diols. The PM3 calculated dihedral angles for *cis*-1,2-cyclopentandiol and *cis*-1,2-cyclohexandiol are approximately 37° and 55°, respectively. These results suggest that *cis*-cyclopentandiol should bind more strongly due to its more planer conformation. Our experimental results are in good agreement with this finding. The differences in interatomic distances of oxygen atoms of hydroxy groups for *cis*-1,2-cyclopentandiol and *cis*-1,2-cyclohexandiol were small.

Table 1. Calculated Dihedral Angles (θ)[a] and Interatomic Distances (d)[b]

Diols	θ / degree			d / Å		
	AM1	PM3	MM2	AM1	PM3	MM2
 *cis*-5	29	37	46 (40-46)[c]	2.7	2.8	2.7
 *cis*-6	52	55	54 (55)[c]	2.8	2.9	2.7
 *trans*-5	68	66	69	3.3	3.3	3.4
 *trans*-6	60	60	61 (62)[c]	2.9	2.9	2.8

[a] Dihedral angle of O−C−C−O. [b] Interatomic distance between two oxygen atom of hydroxy groups. [c] Oshima, K.; Toi, H.; Aoyama, Y. *Carbohydr. Lett.* **1995**, *1*, 223−230.

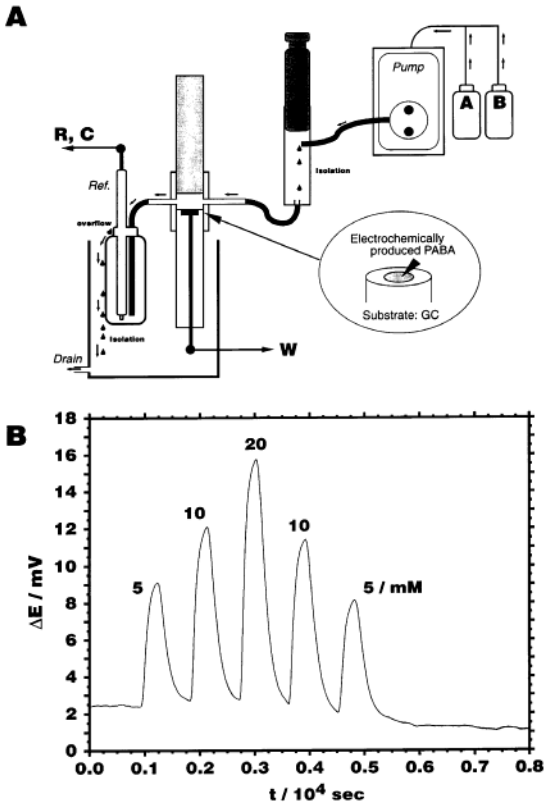


Figure 8. Open circuit potential measurement using flow cell driven by a pump. (A) The experimental setup and (B) the response of D-fructose, 0, 5, 0, 10, 0, 20, 0, 10, 0, 5, and 0 mM at 1 mL min^{-1} in PBS. Each interval was for 10 min and the time required to change the concentration was 10 s. PABA was produced on the GC electrode in the presence of 200 mM NaF.

Thus, ring-size effects do not provide a noticeable change in this distance, and it is not a major factor in observed effects.

Performance under Dynamic Conditions. Since this approach is "reagentless", it is ideally suited to continuous monitoring applications. To demonstrate this capacity, flow injection experiments where performed. D-Fructose was chosen as an analyte due to its higher response and stability. Figure 8 demonstrates the performance of the sensor upon repetitive exposures under various concentrations of D-fructose. The

sensitivity in this configuration was ca. 1 mV mM^{-1} for D-fructose with a background drift of ca. 0.2 mV h^{-1}, corresponding to 0.2 mM h^{-1}. The small contribution of the background drift permitted reliable monitoring of concentrations over 10 h.[77] These preliminary results indicate that the response is reversible and stable and could be applied to monitor other saccharides continuously.

Conclusions

In summary, the complexation of boronic acid group in PABA produces a sufficient change in the pK_a of PABA to result in a significant increase in the electrochemical potential. This behavior provides a selective means for measuring the solution concentration of various sugars and vicinal diols. A Nernstien expression was derived to explain the role of changes in the formal potential and the pK_a on the measured electrochemical potential. These results demonstrate that the boronic acid complexation is well behaved and also suggest that this strategy may be extended to any reversible binding mechanism that changes the pK_a of a redox polymer.

The selectivity for different saccharides can be understood from the relative concentrations of various isomers of particular sugars and the relative binding affinity of boronic acid toward these isomers. In the case of 1,2-cycloalkanediols, calculated geometries indicate that the O−C−C−O dihedral angle and not oxygen atom separation reconciles with known complexation trends. The response profiles observed in this work indicate that the responses are diffusion controlled, although in the case of D-glucose, secondary effects are observed associated with changes in the equilibrium concentrations of the isomers diffusing into the film. It is therefore expected that strategies to enhance mass transport within the polymer films, such as the creation of more porous or composite thin films, will enhance the sensor's response time. Efforts are currently underway to address these possibilities.

The sensitivity and mechanical stability of PABA is enhanced significantly by increasing the fluoride concentration during the electrochemical polymerization. This suggests that eliminating detrimental side reactions that occur at higher potentials is crucial to increasing the sensitivity of the system. Further efforts to increase sensitivity and the dynamic range of the sensor are also under way. Finally, the utility of this sensing strategy was demonstrated in a dynamic environment, indicating that it is suited for the continuous monitoring of saccharides or other diols.

Acknowledgment. This work was supported by the Arnold and Mabel Beckman Foundation.

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(77) In this demonstration, the maximum concentration was arbitrarily limited to 20 mM. Larger concentration ranges are possible.