J. Am. Chem. Soc. **2001,** *123,* 3383−3384

Potentiometric Sensors Based on the Inductive Effect on the pK_a of Poly(aniline): A Nonenzymatic Glucose Sensor

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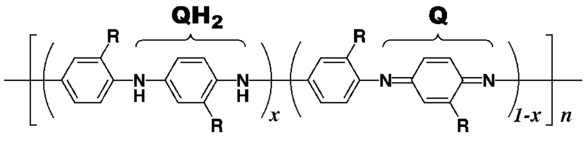
Received December 20, 2000

The use of conjugated polymers for sensor applications continues to be the subject of intense research.[1] In addition to their use as electronic conductors,[2] they have been exploited as active sensing elements by coupling ligands to the backbone. In this approach, binding of an analyte results in physical distortions or changes in electron density, thereby altering conductivity.[1] Poly(aniline),[3] in particular, has received a great deal of attention due to its proton coupled redox chemistry and its resulting pH-dependent properties. For example, poly(aniline) has been used as a pH electrode[4] and has been coupled to reactions that generate or consume protons to create sensors.[5] Herein, we report a new strategy that exploits the inductive effect of reactive substituents on the pK_a of poly(aniline) to produce active sensing elements.

We have focused our investigation on the complexation of diols with boronic acid[6] because: (1) it results in a change in the electron-donating ability of the substituent,[7] (2) it is reversible, and (3) there is considerable interest in using it for glucose sensing.[8] Interest in the area of boronic acid-based sensing strategies[9,10] stems from the need to develop nonenzymatic approaches to glucose sensing. Although the development of an implantable glucose sensor for use in battling diabetes has been the focus of intense research since the 1960s,[11] most advanced glucose sensors are based on glucose oxidase coupled to electrochemical systems.[11,12] Since the response of enzyme-coupled systems is based on the rate of the reaction between the enzyme and glucose, it is inherently sensitive to factors that influence either enzyme activity or glucose mass transport. Boronic acid-based sensors provide an attractive alternative since the complexation is a reversible, equilibrium-based reaction (i.e., the analyte is not consumed).

Poly(aniline) **1** consists of benzenoid diamine and quinone diimine groups[13] where their distribution is a function of the oxidation state of the polymer and the degree of protonation of the polymer is a function of the pH.[14] Since the redox chemistry of **1** involves both electrons and protons, the open circuit voltage, E_{oc}, is sensitive to changes in pH.[4,5] Likewise, it can be shown that the E_{oc} will be a function of K_a. For example, the acid−base reaction associated with the quinone diimine group in **1** can be written as:



1 R=H **2** R=B(OH)$_2$

$$Q + 2H^+ \rightleftarrows QH_2^{\ 2+} \tag{1}$$

with the corresponding acid dissociation constant:

$$K_a = [Q][H^+]^2/[QH_2^{\ 2+}] \tag{2}$$

The reduction of the protonated quinone diimine structure is given by:

$$QH_2^{\ 2+} + 2e^- \rightleftarrows QH_2 \tag{3}$$

with the corresponding Nernst expression:

$$E = E^{o'}_{QH_2^{2+}/QH_2} + (RT/2F)\ \ln[QH_2^{\ 2+}]/[QH_2] \tag{4}$$

It follows that the net proton-coupled redox reaction, (combining eqs 1 and 3) is:

$$Q + 2H^+ + 2e^- \rightleftarrows QH_2 \tag{5}$$

Substituting the expression for $[QH_2^{2+}]$ obtained from eq 2 into eq 4 yields the corresponding Nernst expression for the net reaction, which is a function of K_a:

$$E = E^{o'}_{QH_2^{2+}/QH_2} + (RT/2F)\ \ln[Q][H^+]^2/K_a[QH_2] \tag{6}$$

It then follows that stabilizing QH_2^{2+} by increasing the electron-donating ability of a substituent group, in this case converting boronic acid into the boronate anion complex (Scheme 1),[7] will reduce the K_a of the protonated quinone diimine group (increasing E_{oc}, see eq 6).

The electrochemical polymerization of poly(aniline boronic acid) **2** was achieved by cycling the potential of a glassy carbon electrode between 0 and 1.0 V versus SCE in the presence of 40

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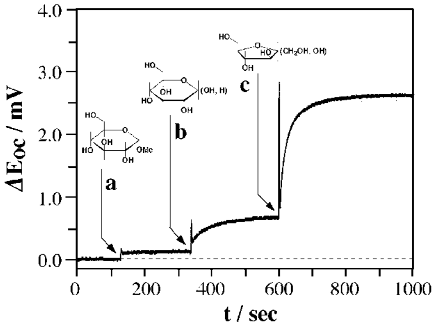
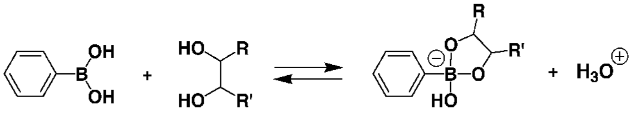


Figure 1. Response curve of a poly(aniline boronic acid) electrode as a function of time upon addition of 6.8 mM: (a) α-Methyl-D-glucoside, (b) D-glucose, and (c) fructose in pH 7.4 PBS.

Scheme 1



mM 3-aminophenylboronic acid, 0.5 M H_2SO_4, 40 mM sodium fluoride[15] and 4 mM Nafion[16] until a final charge of 4.7 mC-cm^{-2} was achieved during the reduction of the film. Scanning was stopped at 1.0 V before being equilibrated in pH 7.4 phosphate buffer saline (PBS) for 24 h. X-ray photoelectron spectra of **2** showed that after equilibrating in PBS, all fluoride was exchanged out of the film.[17] The state of the polymer was then followed by monitoring E_{oc} versus SCE in PBS. The inherent selectivity of the boronic acid complexation reaction (Scheme 1) to the nature of the diol is illustrated in Figure 1 by the change in the E_{oc} upon the addition of the same concentrations of different sugars. The difference in sensitivity qualitatively follows the difference in binding constants with phenylboronic acid reported in neutral aqueous solutions (fructose > glucose[18] > α-methyl-D-glucoside).[19]

Due to the sensitivity of **1** to pH,[4,5] it is expected that the E_{oc} will be sensitive to the local pH drop within the film associated with the formation of the complex (Scheme 1). This was confirmed by observing increases in E_{oc} for **1** coated with poly(vinylphenylboronic acid) upon the addition of glucose (see Supporting Information). However, as expected, the responses associated with local pH changes were transient in nature due to the rapid return to equilibrium with the bulk solution. Similar transient increases in E_{oc} are observed as spikes in Figure 1 upon the addition of different sugars.

The slower steady-state responses that are observed in Figure 1 are consistent with a change in pK_a of **1** and subsequent protonation. It should be noted that the positive change in E_{oc} is in contrast to the change in redox behavior expected for the inductive effect on an isolated redox center that does not involve the transfer of protons. For example, according to the results obtained with the boronic acid substituted ferrocene/ferrocenium redox couple, the apparent formal potential decreases[10b] upon formation of the electron-donating boronate anion structure.[7]

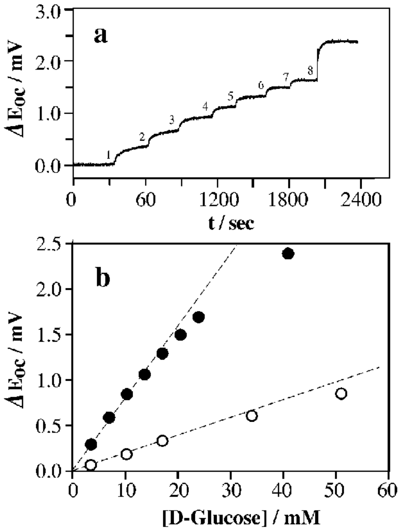


Figure 2. (a) D-Glucose response curve of a poly(aniline boronic acid) electrode in pH 7.4 PBS as a function of time. Additions resulted in the following series of concentrations: (1) 3.4, (2) 6.8, (3) 10.2, (4) 13.6, (5) 17.0, (6) 20.4, (7) 23.8, and (8) 40.8 mM. (b) Calibration curves of D-glucose (filled circles) for a poly(aniline boronic acid) electrode and of D-glucose (open circles) for poly(aniline)-coated electrode in pH 7.4 PBS.

The use of this sensor design strategy is shown in Figure 2 where a typical response profile and calibration curve is shown for glucose along with the control. A stepwise increase in E_{oc} was observed upon addition of glucose. A control experiment using only **1** exhibited similar increases in E_{oc}; however, the total shift of the potential was clearly smaller. Reversibility of the sensor was confirmed by the return of the E_{oc} to its initial value upon exposure to blank PBS.

In summary, we report a novel sensing approach exploiting inductive effects on the pK_a of poly(aniline) and in turn its electrochemical potential. This strategy was demonstrated using boronic acid chemistry to produce a nonenzymatic glucose sensor. The selectivity of the boronic acid complexation reaction toward different sugars was reflected in the sensitivity of the E_{oc} to various sugars. Finally, calibration curves for D-glucose obtained in PBS (pH 7.4) demonstrate that reversible responses can be obtained within the physiological relevant range 4−6 mM using this approach. Research into the use of this strategy with other systems is currently underway.

Acknowledgment. We thank Professor Frances Arnold and Dr. Alan Michaels for helpful discussions. This work is dedicated to the memory of Dr. Alan Michaels.

Supporting Information Available: Detailed experimental procedures, spectroscopic data, and supporting experimental data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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