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Using an Indicator Displacement Assay to Monitor Glucose Oxidase Activity in Blood Serum

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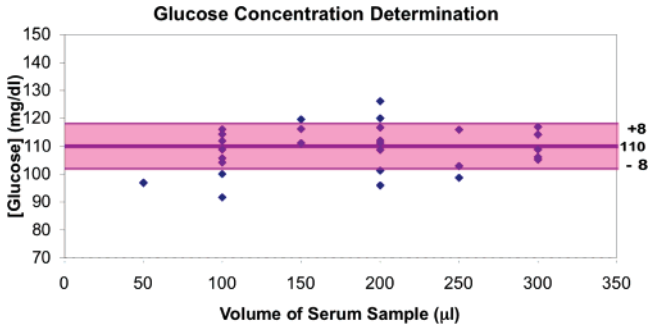
ABSTRACT



Using a boronic acid receptor that was previously found to have high affinity for gluconic acid, we created a colorimetric indicator displacement assay (IDA) that can report the concentration of the product of glucose oxidase (GOx) catalyzed glucose oxidation. The color change obtained directly reflects the concentration of glucose. Our sensing ensemble was then successfully applied to determine the glucose concentration in human serum, which offers a facile, colorimetric, sensitive, and accurate glucose test.

Glucose sensing continues to be an active area of research because of the increasing number of individuals diagnosed with diabetes.[1] The traditional commercial glucose test is electrochemical, involving an amperometric electrode where the glucose concentration is monitored by a change in current flow caused by the glucose oxidase (GOx) catalyzed production of hydrogen peroxide, or sometimes by the consumption of oxygen.[2] In this method, the generated hydrogen peroxide is oxidized under a constant working potential, and the extent of oxidation corresponds to the glucose concentration. However, it needs careful calibration because other oxidizable components may interfere at the working potential. Acid-sensitive polymers have alternatively been used.[3] In this method, GOx generates gluconic acid which lowers the pH and causes shrinking of the polymer. A vibrational frequency change correlates to the glucose concentration. The detecting process is reversible, but the sensitivity will dramatically decrease with increasing ionic strength. Optical methods have also been reported, along with their potential

(1) (a) Pickup, J.; McCartney, L.; Rolinski, O.; Birth, D. *BMJ* **1999**, *319*, 1−4. (b) Fang, H.; Kaur, G.; Wang, B. *J. Fluoresc.* **2004**, *14*, 481−489. (c) Koschinsky, T.; Heinemann, L. *Diabetes Metab. Res. Rev.* **2001**, *17*, 113−123. (d) Moschou, E. A.; Sharma, B. V.; Deo, S. K.; Daunert, S. J. *Fluorescence* **2004**, *14*, 535−547. (e) Ramamoorthy, R.; Dutta, P. K.; Akbar, S. A. *J. Mater. Sci.* **2003**, *38*, 4271−4282. (f) Wang, J. *Sens. Update* **2002**, *10*, 107−119.

(2) (a) Pereira, C. M.; Oliveira, J. M.; Silva, R. M.; Silva, F. *Anal. Chem.* **2004**, *76*, 5547−5551. (b) Heller, A. *Annu. Rev. Biomed. Eng.* **1999**, *01*, 153−175. (c) Wu, J.; Qu, Y. *Anal. Bioanal. Chem.* **2006**, *385*, 1330−1335. (d) Gao, Z.; Xie, F.; Shariff, M.; Arshad, M.; Ying, J. Y. *Sens. Actuators, B* **2005**, *B111*−*B112*, 339−346. (e) Lawrence, N. S.; Deo, R. P.; Wang, *J. Anal. Chem.* **2004**, *76*, 3735−3739. (f) Li, J.; Chia, L. S.; Goh, N. K.; Tan, S. N.; Ge, H. *Sens. Actuators, B* **1997**, *B40*, 135−141. (g) Granot, E.; Basnar, B.; Cheglakov, Z.; Katz, E.; Willner, I. *Electroanalysis* **2006**, *18*, 26−34. (h) Wolfbeis, O. S.; Oehme, I.; Papkovskaya, N.; Klimant, I. *Biosens. Bioelectron.* **2000**, *15*, 69−76.

(3) Cai, Q.; Zeng, K.; Ruan, C.; Desai, T. A.; Grimes, C. A. *Anal. Chem.* **2004**, *76*, 4038−4043.

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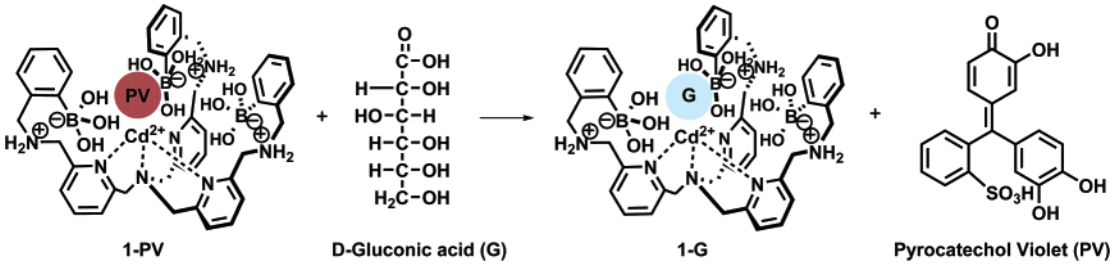
Scheme 1. IDA Application in Gluconic Acid Association



applicability in vivo for noninvasive detecting and high sensitivity.[4] Asher has reported a crystalline colloidal array embedded polyacrylamine hydrogel that responds to low concentrations of glucose by a Bragg diffraction change from greenish blue to red.[5] Further, the Shear group applies horseradish peroxide to uptake the GOx product H_2O_2, and the generated O_2 is caught by 4-aminoantipyrine and 4-hydroxybenzoic acid to generate quinoneimine, a bright red dye. Via the absorbance change, the concentration of glucose can be determined in various drinks.[6] We wanted to extend the use of indicator displacement assays (IDA), which have been successfully applied in various media using sensitive colorimetric dyes. Here, we report the application of an IDA method to glucose sensing in serum, creating an attractive assay of potential relevance to diabetes.

We previously reported a metalated boronic acid receptor (**1**) that shows excellent selectivity and high affinity to gluconic acid with a stoichiometry of 1:1 and a Ka of 5.6×10^6 M^{-1}.[7] We now report the use of **1** in a sensing assay. A colorimetric response to gluconic acid was created using an indicator displacement assay in 3:1 (v/v) MeOH/H_2O at neutral pH. Pyrocatechol violet (PV) was chosen as the indicator (Scheme 1), and a 1:1 (molar ratio) of **1**/PV solution was prepared, giving a purplish red color. Upon introduction of the gluconic acid, because of its stronger association to receptor **1**, PV was displaced from the receptor pocket, giving the free indicator color, yellowish green. A large spectral response is found for gluconic acid, demonstrating that we could monitor this product of GOx. As shown in Figure 1, little to no displacement of the indicator occurs upon the addition of glucose. This demonstrates that we could detect the gluconic acid in the presence of glucose or other sugars.

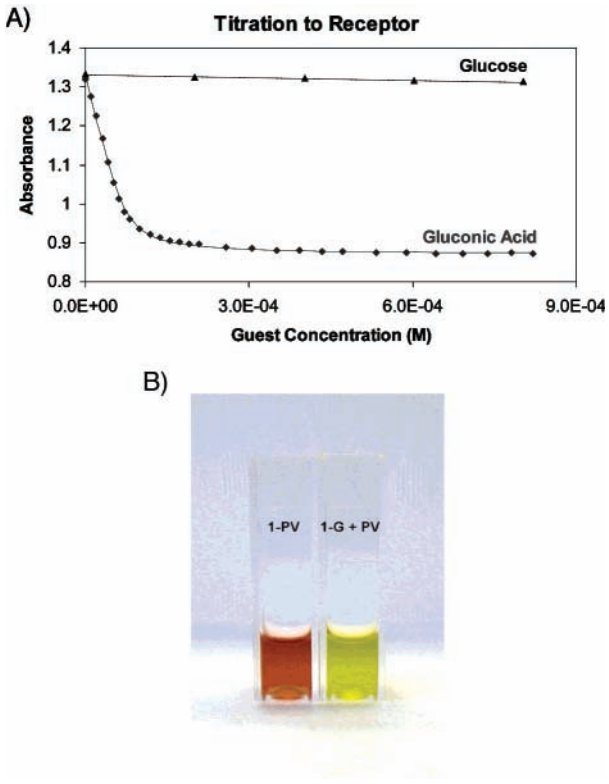


Figure 1. (A) UV/vis titration results, where [**1**] = 0.13 mM and [PV] = 0.13 mM in MeOH−H_2O 3:1 (v/v). (B) Addition of gluconic acid to receptor **1** causes a displacement of PV from **1**, resulting in a large color change. [**1**] = 0.13 mM, [PV] = 0.13 mM, and [G] = 0.13 mM, all in MeOH−H_2O 3:1 (v/v).

Using this IDA and the fact that gluconic acid was the only product generated with GOx, we explored the determination of gluconic acid concentrations in blood. Because the reaction generates hydrogen peroxide, the enzyme catalase was applied to sequester the H_2O_2 (Scheme 2). The H_2O_2 was sequestered because boronic acid based receptors have previously been shown to react with H_2O_2.[8]

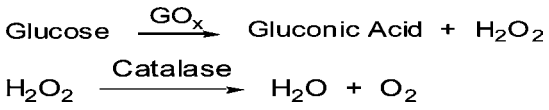
(4) (a) Pickup, J. C.; Hussain, F.; Evans, N. D.; Rolinski, O. J.; Birch, D. J. S. *Biosens. Bioelectron.* **2005**, *20*, 2555−2565. (b) Yoon, J.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 5874−5875. (c) Antwerp, V.; Peter, W.; Mastrototaro, J. J. PCT Int. Appl., 1997, *58*, WO 9719188. (d) James, T. D.; Shinkai, S. *Top. Curr. Chem.* **2002**, *218*, 159−200. (e) DiCeswere, N.; Pinto, M. R.; Schanze, K. S.; Lakowicz, J. R. *Langmuir* **2002**, *18*, 7785−7787. (f) Ye, K.; Schultz, J. S. *Anal. Chem.* **2003**, *75*, 3451−3459. (g) Shiomi, Y.; Miwako, S.; Tsukagoshi, K.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2111−2117. (h) Zhang, L.; Small, G. W. *Anal. Chem.* **2002**, *74*, 4097−4108. (i) Berger, A. J.; Koo, T.; Itzkan, I.; Korwitz, G.; Feld, M. S. *Appl. Opt.* **1999**, *38*, 2916−2926. (j) Shafer-Pektuer, K. E.; Haynes, C. L.; Glucksberg, M. R.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2003**, *125*, 588−593. (k) Luebbers, D. W.; Opitz, N. *Sens. Actuators* **1983**, *4*, 641−654. (l) Fang, H.; Kaur, G.; Wang, B. *J. Fluoresc.* **2004**, *14*, 481−489. (m) Pringsheim, E.; Terpetschig, E.; Piletsky, S. A.; Wolfbeis, O. S. *Adv. Mater.* **1999**, *11*, 865−868. (n) Thoniyot, P.; Cappuccio, F. E.; Gamsey, S.; Corders, D. B.; Wessling, R. A.; Singgaram, B. *Diabetes Technol. Therapeutics* **2006**, *8*, 279−287.

(5) Asher, S. A.; Alexeev, V. L.; Goponenko, A. L.; Sharma, A. C.; Lednev, I. K.; Wilcox, C. S.; Finegold, D. N. *J. Am. Chem. Soc.* **2003**, *125*, 3322−3329.

(6) Curey, T. E.; Salazar, M. A.; Oliveira, P.; Javier, J.; Dennwas, J.; Rao, P.; Shear, J. S. *Anal. Biochem.* **2002**, *303*, 42−48.

(7) Zhang, T.; Anslyn, E. V. *Org. Lett.* **2006**, *8*, 1649−1652.

Scheme 2. Enzyme-Catalyzed Glucose Oxidation



To optimize the reaction conditions, several variables were examined: the [GOx], [catalase], pH, and reaction time (Supporting Information). The optimization led us to prepare a series of 5 mL aqueous solutions at pH ~ 7 as standards, each of which contained 30 μL of GOx (1 mg/mL), 200 μL of catalase (1 mg/mL), 50 mM Tris buffer, and 0−140 μL of a glucose solution (50.23 mM), resulting in glucose sensitivity between 0 and 1.41 mM. This concentration range was targeted because the procedure ultimately used with blood involves a dilution (see below).

The oxidation commences upon the addition of different amounts of glucose to the 5 mL aliquot containing GOx and catalase. Aliquots of 250 μL of this solution were removed from the reaction and mixed with a 750 μL MeOH solution containing receptor **1** (0.13 mM) and an indicator (pyrocatachol violet, 0.13 mM). The solutions were analyzed by UV−vis spectroscopy, and the absorbance was correlated to gluconic acid concentrations, giving a calibration curve (Figure 2). This curve was then used to analyze for glucose in crude blood samples.

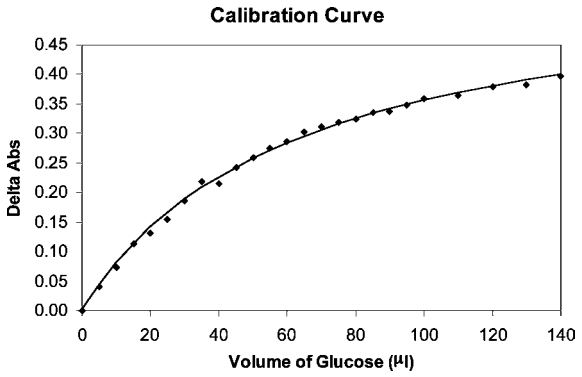


Figure 2. Glucose calibration curves. [Glucose] = 50.34 mM.

Human serum (glucose 110 mg/dL, Sigma-Aldrich) was analyzed. With the enzyme and buffer solutions described above, instead of using glucose, the human serum was directly applied in varying amounts (50, 100, 150, 200, 250, 300 μL). We used a series of concentrations because patients with diabetes routinely withdraw varying volumes that are not necessarily controlled. The sample treatment procedure was the same as that in the calibration experiment. The absorbance was recorded and converted to glucose concentration using the calibration curve (mg/mL) (Figure 2). For each addition of serum, the glucose concentration was determined. In each analysis, the concentration was that quoted by Sigma-Aldrich with an average ± 7% error: [glucose] = 110 ± 8 mg/dL (Figure 3). Hence, this colorimetric methodology is accurate for determining glucose concentration in human blood at a variety of different blood volumes.

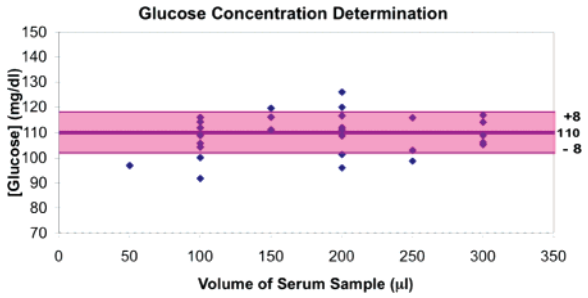


Figure 3. Glucose determination in human serum. All samples were 110 mg/dL of glucose, and several tests were performed for different volumes of the serum. Each test was within 7% of the correct glucose value.

In conclusion, using a receptor that was previously found to have high affinity to gluconic acid, we created a colorimetric IDA that can report the concentration of the product of GOx catalyzed glucose oxidation. The color change obtained directly reflects the concentration of glucose. Our sensing ensemble was then successfully applied to determine the glucose concentration in human serum, which offers a facile, colorimetric, sensitive, and accurate glucose test.

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Supporting Information Available: A PDF file of condition optimization. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Wu, S.; Waugh, W.; Stella, V. J. *J. Pharm. Sci.* **2000**, *89*, 758−765.