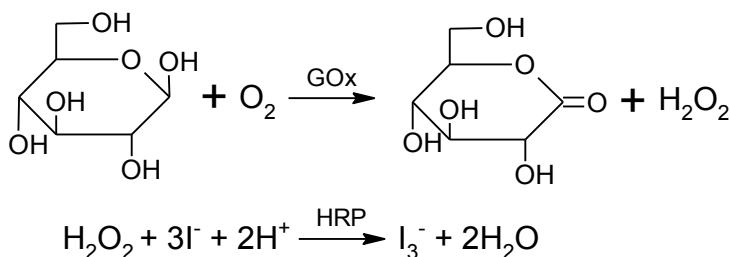


## Glucose Determination in Urine by Paper Microfluidics

Paper-based microfluidics has the potential to bring inexpensive, simple point-of-care (POC) to isolated locations throughout the world.<sup>1</sup> In many cases, the procedure will act as a screening method helping determine what patients would require further testing. In this experiment, you will use paper-based microfluidics as a non-invasive, fast, inexpensive urine screening method for diabetes.<sup>2</sup> You will prepare a series of standards for glucose in (artificial) urine and screen a standard patient (with known urine glucose concentration) and an unknown patient for diabetes.

### Color Forming Reagent

Glucose will be detected and quantified using a colorimetric biosensor. This detection method involves the glucose oxidase (GOx) catalyzed oxidation of glucose into gluconic acid and hydrogen peroxide which then oxidizes iodide (colorless) to triiodide (brown) as catalyzed by horseradish peroxidase (HRP) (Figure 1). The relative intensity of the brown/orange color of the triiodide can be used to determine the original concentration of glucose in the solution.



**Figure 1.** Glucose oxidase (GOx) catalyzed oxidation of glucose to gluconic acid and hydrogen peroxide followed by horseradish peroxidase (HRP) catalyzed oxidation of colorless iodide to brown triiodide

### Materials and Equipment

Artificial Urine (AU) (see reference 1 for composition)

25.0 mM glucose standard (diluted in AU) (record exact concentration)

Glucose testing solution

5:1 glucose oxidase:horseradish peroxidase, 0.6 M KI, in pH 6.0 buffer (distributed in a centrifuge tube – use only what you need dispensing with a clean micropipet tip)

Standard patient AU (record concentration)

Unknown patient AU (record unknown number)

Microcentrifuge tubes (graduated, 2 mL maximum volume)

Various micropipets

Chromatography paper

Device template(s)

Pencil and wax pencil

Large beaker

Oven

<sup>1</sup> Martinez, A.W.; Phillips, S.T.; Carrilho, E.; Thomas III, S.W.; Sindi, H.; Whitesides, G.M. "Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis." *Anal. Chem.* **2008**, *80*, 3699-3707.

<sup>2</sup> Adapted from Gross, E.M.; Clevenger, M.E.; Neuville, C.J.; Parker, K.A. "A Bioanalytical Microfluidics Experiment for Undergraduate Students" in *Teaching Bioanalytical Chemistry*; Hou, H.J.M., Ed; ACS Symposium Series 1137; American Chemical Society: Washington, DC, 2013; pp 85-104.

### Standard preparation and sample testing

1. From a 25.0 mM stock glucose solution, make 1000  $\mu\text{L}$  (1 mL) each of 5 standard glucose solutions with concentrations ranging from 0.17 mM to 3.0 mM. The middle standard should have a concentration of 0.8 mM (the normal maximum non-fasting glucose concentration in urine). It may be advantageous to make the lower concentration(s) using serial dilution from the higher concentration standards. Use micropipets to deliver the correct volumes of the standards (no less than 10  $\mu\text{L}$  each) and dilute to 1.00 mL in the provided graduated centrifuge tubes. Each solution should be diluted to final volume using artificial urine. (Why is it important that standards are prepared using artificial urine?)
2. Follow the directions for device fabrication in the file titled “Paper-based Microfluidics as an Alternative to Spectrophotometry.” You will want to prepare four different devices for testing. (Three will be used for standards and a blank while the fourth will be used for your patients. Why is it important to have multiple tests for each analysis?)
3. Using a micropipet, deposit 40  $\mu\text{L}$  of the GOx/HRP/KI glucose testing solution in the center well of each device and allow it to flow down the arms to the testing zone of each arm. To appropriately transfer the solution, touch the tip of the pipet to the paper and allow it to begin wicking the fluid, then, slowly expel the fluid. Very gently push the second button on the pipet that expels the last drop (be careful not to splash the solution). Allow at least 10 minutes for the device to dry in the dark at room temperature.
4. Standard devices: Using a micropipet, spot 2  $\mu\text{L}$  of a standard into each glucose test zone. The solution should completely fill the circle of the test zone and not fill in the channel. Of the six test zones on each standard device, five should contain standards (labeled 1-5 outside of the wax) and the sixth should be a “blank” (just artificial urine).
5. Patient device: Using a micropipet, spot 2  $\mu\text{L}$  of your patients’ urine samples into each glucose test zone. The solution should completely fill the circle of the test zone and not fill in the channel. Of the six test zones, three should contain urine from your standard patient and three from your unknown patient. (Make sure to label next to each zone.)
6. Allow the devices to dry and react for 10 minutes at room temperature. Follow the directions in “Paper-based Microfluidics as an Alternative to Spectrophotometry” and “Using ImageJ for Data Analysis” to analyze your devices.

### Data Analysis

Use Minitab to create a calibration curve for the results of your glucose analysis. Use the macros discussed in the Minitab calibration handout (see glassware lab manual files) to find the uncertainties of the slope and intercept for your curve. Determine the concentration of glucose in the urine samples from your two patients. Use the “unknown” macro in Minitab to find the 95 % confidence intervals of your results. Follow the directions at the end of the handout to show these confidence intervals on your calibration curve in Minitab. Using these results, determine whether your “unknown” patient should be further screened for diabetes.

In your conclusions, discuss the accuracy and precision of your test. Use the “standard” patient to discuss the validity of the results for your unknown patient. Using the results of your blank analysis, find the concentration limit of detection for your device. Use your calibration curve to estimate a linear dynamic range for the device. Discuss how these results could affect your ability to accurately screen for diabetes. Given the information you have gathered about this measurement technique, what can you say about its value as a screening method? A

screening method is designed to be a broadly applicable method used to determine which patients may require more expensive, often invasive, examinations to more accurately and precisely diagnose disease status. It may have a somewhat high rate of false positive results (indicates possible disease when none is present requiring further diagnostic tests), but should rarely give false negative results (indicates no disease when disease actually is present).

In a previous experiment in this course, you determined the concentration of glucose in a sugar tablet using an oxidation-reduction titration method. Compare the results of that procedure with this one. In your discussion, consider the relative concentrations of glucose in each experiment. What advantages does each method have over the other? For what applications would each be appropriate?