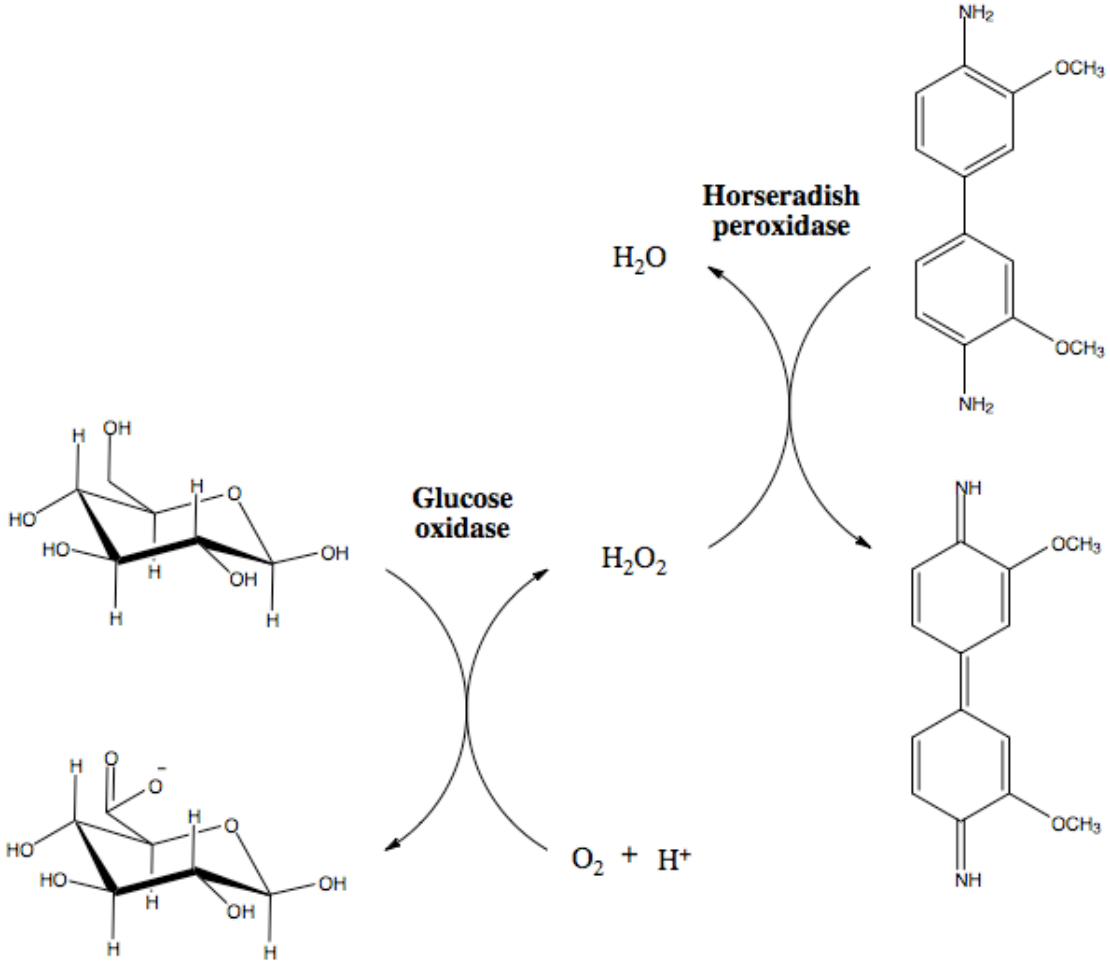
Glucose Concentration Determination

Adapted by T. Gries from Clinical Laboratory Methods, 8th Edition (1974)

Blood glucose concentration is highly regulated. While quick electrochemical meters that measure blood glucose levels are commercially available, assays with much great sensitivity and reproducibility are used in the clinical laboratory setting (nowadays these assays are mostly automated). We are going to use the Glucose oxidase method to determine the glucose concentrations of several unknowns and from freshly drawn mouse blood.

The glucose oxidase assay couples the activity of glucose oxidase to horseradish peroxidase. The observable parameter for this assay is the end product derivative of o-dianisidine dye which after quenching each reaction will have an absorbance maximum at 540 nm. Below is a schematic of the assay reaction:



Before Lab:

1. Copy the reaction scheme into your notebook and clearly indicate whether each arcing arrow is an oxidation or reduction reaction.
2. Use the USDA Nutritional Database to determine the concentration of glucose in raw tangerines in units of mg glucose per g of tangerine.
3. Make a table that indicates the amount of glucose or fructose and phosphate buffer that you will add to tubes 1 through 9.
4. Describe what you expect to observe for the results of the glucose assay for tubes 1-5.
5. Describe what you expect to observe for the results of the glucose assay for tubes 7-9.

Additional Materials:

- 0.1 M Potassium phosphate buffer; pH 7.2
- 12.5 U/mL glucose oxidase + 2.5 U/mL peroxidase in 0.1 M potassium phosphate buffer; pH 7.2
- 0.5 mg/mL D-glucose in 0.1 M potassium phosphate buffer; pH 7.2
- 0.5 mg/mL D-fructose in 0.1 M potassium phosphate buffer; pH 7.2
- 30% H_2SO_4
- Tangerine slices

Extracting glucose from a food source

Record the mass of a small piece of tangerine (0.05 g) in a weigh boat. Grind the slice using a mortar and pestle being sure to collect all of the tangerine material from the weigh boat and keep all the material in the mortar. Add 1 mL of 0.1 M potassium phosphate and continue grinding. Place all of the food mash into a 1.7 mL microfuge tube and spin balanced tubes for 2 minute at 14,000 rpm to pellet solids. Use an adjustable pipette to estimate the total volume of supernatant.

Preparing assays

Use 0.1 M potassium phosphate to make all solutions!

Prepare the following solutions in test tubes to create a calibration curve:

Tube	**[Glucose] (mg/mL)**	**$Volume_{final}$ (mL)**
1 (blank)	0	0.25
2	0.01	0.25
3	0.02	0.25
4	0.04	0.25
5	0.06	0.25
6	0.08	0.25

Prepare the following solutions in test tubes to test the specificity of this assay:

Tube	**[Fructose] (mg/mL)**	**$Volume_{final}$ (mL)**
7	0.04	0.25
8	0.06	0.25
9	0.08	0.25

Prepare the following solutions in test tubes to determine the [glucose] in your unknown samples:

Tube	Sample dilution	$Volume_{final}$ (mL)
10 (*)	supernatant (65 uL)	0.25
11	supernatant (65 uL)	0.25
12	unknown	0.25

The (*) solution is prepared to correct for any coloration due to pigments extracted from the food along with the glucose [see below].

Starting Assays

Add 0.5 mL of the 12.5 U/mL glucose oxidase + 2.5 U/mL peroxidase solution to tube #1. Cover the tube with parafilm and invert several times to mix. Take note of the time. Start the reaction in each tube following the same method except tube 10 to which 0.5 mL of 0.1 M potassium phosphate should be added in lieu of the enzyme solution.

Once each reaction has been started, transfer the covered tubes to a 37 C water bath for thirty minutes.

Quenching and Analyzing Reactions

Place solutions on ice for a few minutes. To quench each reaction, very carefully add 0.5 mL of 30% H_2SO_4 to each tube, cover with parafilm and invert to mix.

Allow each reaction to cool to room temperature. Use the spectrophotometer to measure the absorbance of each reaction at 540 nm. Use Tube 1 as a blank [be sure to also record the absorbance of Tube 1]. If the absorbance is greater than 1.5, dilute the reaction with solution of 0.06 M potassium phosphate + 12% H_2SO_4.

Questions

1. Staple in a well-annotated table that summarizes your absorbance data results.

2. Staple in a plot of absorbance versus [glucose] in mg mL^{-1} for your standard glucose solutions. There is no need to perform any dilution calculations here since all the reactions were treated identically. The x-axis should be the concentration of glucose in the 0.25 mL. There should be a linear correlation of the data. Record the equation of the line with the correlation coefficient.

3. Determine the standard deviation in x-values.

4. Use the calibration curve to determine the [glucose] in each test solution. Staple in a well-annotated table to report the results of the specificity tests (Tubes 7-9) and food source tests (Tubes 10-12) in mg/mL. Include errors.

5. How specific is this glucose assay for glucose? Explain your reasoning quantitatively.

6. Do you have concerns about other molecules in tangerines interfering with your absorbance assay? Explain your reasoning quantitatively.

7. How many mg of glucose did you extract from the small tangerine piece? Include error.

8. What is the concentration of glucose in mg glucose per g of tangerine? Include error.

9. How does your determination for glucose concentration in tangerines compare to the USDA Nutritional database?