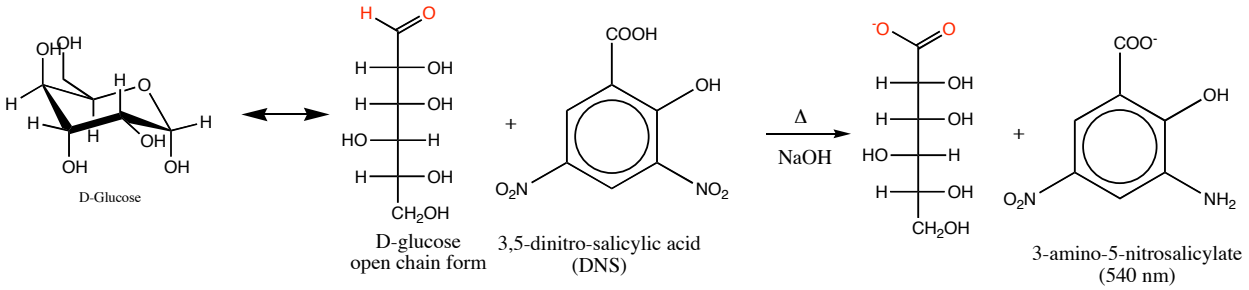
CHEM 4401 Biochemistry I Laboratory

Analysis of Reducing Sugars in Beverages

Reading assignment: Lehninger (5th Ed.) Ch 7.1: 235-244

Carbohydrate are essentially aldehydes or ketones that contain multiple hydroxyl (-OH) groups. The most common monosaccharides (single carbohydrate molecules) found in nature contain either 5 or 6 carbons. Though they exist primarily as cyclic molecules (pyranoses and furanoses), monosaccaride chains do open, revealing in more detail the presence and location of the aldehyde and ketone functional groups:



The free aldehyde groups on the open-chain forms of monosaccharide can serve as mild reducing agents. We can take advantage of this fact to quantitatively measure the amount of sugars in solution. The alkaline DNS test for reducing sugars is simple, fast and reliable and was traditionally used in the medical field for the determination of sugar levels in the blood and urine. Reducing 3,5-dinitro-salicyclic acid forms a colored product, 3-amino-5-nitrosalicylate, that absorbs light with a wavelength of 540 nm. We will use this property to determine the carbohydrate content in a number of beverages. As the reagent only works with the aldehyde groups on free sugars, we will first hydrolyze our beverages with acid to break down any di- or oligosaccharides present. Glucose solutions of known concentration will also be analyzed to construct a standard curve.

Experimental Procedure

Hydrolysis of Di- and Oligosaccharides

1. Pipet 0.3 ml of each beverage (diluted 1:25) into a labeled test tube
2. Add 1 ml of 1.5 M Sulfuric acid to each tube. Do NOT use a pipettor. Mix gently using a vortexer at a low speed setting.
3. Heat in a boiling water bath for 30 minutes.
4. Cool the tubes in an ice bath for 1 minute.
5. **Carefully** add 1.2 ml of 10% NaOH to each tube. Mix gently.
6. Add 0.5 ml of distilled H_2O to each tube. This will produce a final volume of 3 ml. Mix.

DNS Assay

1. Prepare the following samples in test tubes. Mix gently.

		Tube					Beverage		
Component (ml)	**Blank**	**1**	**2**	**3**	**4**	**5**	**No. 1 6**	**No. 2 7**	**No. 3 8**
Distilled Water	3	2	2	2	2	2	2	2	2
Glucose Std **A** (200 ug/ml)	-	1	-	-	-	-	-	-	-
Glucose Std **B** (400 ug/ml)	-	-	1	-	-	-	-	-	-
Glucose Std **C** (800 ug/ml)	-	-	-	1	-	-	-	-	-
Glucose Std **D** (1000 ug/ml)	-	-	-	-	1	-	-	-	-
Glucose Std **E** (1200 ug/ml)	-	-	-	-	-	1	-	-	-
Beverage Hydrolysate	-	-	-	-	-	-	1	1	1
DNS reagent	1	1	1	1	1	1	1	1	1

2. Place all tubes in a boiling water bath for *10 minutes*.
3. Cool tubes in an *ice bath* for 1 minute.
4. Mix gently using a vortexer.
5. Read the absorbance of all sample at 540 nm. Be sure to blank your spectrophotometer first.

Waste

Transfer contents of sample tubes to an appropriate waste container

Presentation of Data

1. Prepare a standard curve by plotting the absorbance at 540 nm on the ordinate (y-axis) and the amount of glucose in the standards on the abscissa (x-axis). Prepare a line of best fit to your data. Determine the equation for your line ($y = mx + b$). Use the equation to calculate the concentration of glucose in your *test tube* samples.
    a. Figure legend with appropriate description (2 pt)
    b. Graph with regression line (2 pt)
    c. Axes labeled with appropriate units (2 pt)
    d. Equation for regression line (2 pt)
    e. Concentration of glucose in <u>each</u> beverage <u>hydrolysate test tube sample</u> (calculated using regression line equation) reported in figure legend (remember units) (3 pt).
2. Calculate the concentration of glucose in <u>each</u> of the *undiluted beverages*. Be sure to account for the initial dilution of the beverage (see step 1 of **Hydrolysis of Di- and Oligosaccharides**), as well as the dilution that occurred during this part of the procedure. Prepare a table that reports these values. Be sure to include a title and appropriate units.
    a. Table numbered with appropriate description (1 pt)
    b. Glucose values reported for each beverage with appropriate units (3 pt)
3. Laboratory performance (preparation, safety, attention to detail, effort, clean up) 2 pt.