Experiment 2

Observing the Reaction Kinetics of Sucrose with Polarimetry

Polarimeters can be used in kinetics experiments to follow the change in concentration of an optically active sample as a reaction proceeds. Sugars are common examples of optically active compounds. Sucrose is a disaccharide that can be broken down into its two substituent monosaccharides, glucose and fructose.

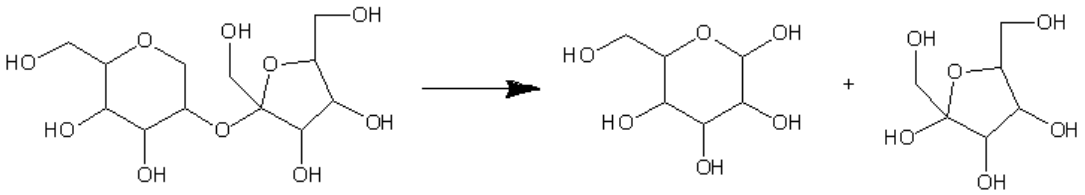

Figure 1 The breakdown of sucrose

This process occurs too slowly in water to be monitored on any real time scale, so a catalyst, acid or enzyme, must be added to accelerate the reaction rate. In this experiment, hydrochloric acid is used to catalyze the reaction while its rate is monitored using a polarimeter. The experiment will be repeated using the enzyme invertase to catalyze the reaction.

The reaction rate is going to be monitored by the change in concentration of the starting material, sucrose. Concentration is proportional to the observed optical rotation of the sample, as determined by the polarimeter, according to Biot's law

$$\alpha = [\alpha]\, \ell\, c$$

where α is the observed optical rotation in units of degrees, $[\alpha]$ is the specific rotation in units of degrees (the formal unit for specific rotation is degrees dm^{-1} mL g^{-1}, but scientific literature uses just degrees), ℓ is the length of the cell in units of decimeters (dm), and c is the sample concentration in units of grams per milliliter (g/mL).

OBJECTIVES

- Calculate the specific rotation of sucrose using a polarimeter.
- Observe the cleavage kinetics of sucrose with an acid catalyst, hydrochloric acid.
- Observe the cleavage kinetics of sucrose with an enzyme catalyst, invertase.
- Calculate the rate constant for each run from the rotational readings.

MATERIALS

One of the following
- Chromebook, computer, **or** mobile device with Vernier Instrumental Analysis app[1]
- LabQuest 2 (software is pre-installed; v2.8.7 or newer required[2])
- LabQuest 3 (software is pre-installed; v3.0.3 or newer required[2])

Go Direct Polarimeter
polarimeter sample cell
100 mL and 25 mL volumetric flasks
100 mL beaker
6 M hydrochloric acid (HCl)
sucrose
invertase from baker's yeast (*S. cerevisiae*)

PROCEDURE

Part I Specific rotation of sucrose

1. Obtain and wear goggles. Protect your arms and hands by wearing a long-sleeve lab coat and gloves. Conduct this reaction in a fume hood.

2. Accurately prepare 100 mL each of a 30% (w/v) and a 15% (w/v) sucrose solution.
   Caution: *Treat all laboratory chemicals with caution. Prudent laboratory practices should be observed*.

3. Set up the Go Direct Polarimeter by following the directions for your equipment:
   - Instrumental Analysis: Launch Instrumental Analysis. Connect the Go Direct Polarimeter to your Chromebook, computer, or mobile device.
   - LabQuest: Connect the Go Direct Polarimeter to your LabQuest 2 or LabQuest 3.

4. Calibrate the polarimeter.
   a. Pour distilled water in the polarimeter cell to a height of 10 cm. It is important to read the height to the nearest 0.1 cm. Read to the bottom of the meniscus.
   b. Place the cell in the polarimeter, then follow the appropriate steps:
      - Instrumental Analysis: Click or tap Finish Calibration. When the polarimeter is ready, click or tap Done.
      - LabQuest: Select Calibrate from the Sensors menu. Tap Calibrate Now and follow the instructions on the screen. When the polarimeter is ready, tap OK.

5. You are now ready to add the optically active sample into the polarimeter cell.
   a. Rinse the polarimeter cell with a few milliliters of 15% sucrose solution. Pour the sample in the polarimeter cell to a height of 10 cm. Record this value to the nearest 0.1 cm in the correct data table.
   b. Place the sample cell in the polarimeter.

[1]Instrumental Analysis v1.2 or newer required; download the most recent version for free at www.vernier.com/ia

[2]Download the most recent version of LabQuest software at www.vernier.com/downloads

c. Start data collection. Data collection will stop automatically.

d. Store the data, if necessary:
   - Instrumental Analysis: Data are stored automatically. Continue to the next step.
   - LabQuest: To store the data, tap the File Cabinet icon. Then, continue to the next step.

6. Use the Statistics or Curve Fit tool to determine the angle closest to 0° where the illumination is at a maximum. This is the observed angle of rotation of the plane of polarized light for the optically active sample. Record this value in Table 1.

   To access the Statistics or Curve Fit tools, follow the appropriate steps:

   - Instrumental Analysis: Highlight the peak of interest, if applicable. Then, click or tap Graph Tools, .
   - LabQuest: Highlight the peak of interest, if applicable. Then, tap Analyze.

7. Repeat Steps 5–6 two more times for the 15% sucrose solution and a total of three times for the 30% sucrose solution. Each time, empty the sample cell and rinse it with a small amount of the next solution you will be testing. **Tip**: You can reuse your solutions in the following experiments.

Part II Kinetics of sucrose with acid

8. Change the polarimetry mode to Kinetics.
   - Instrumental Analysis: In the Polarimetry Settings menu, select Kinetics as the Polarimetry Mode. Then change the rate to 1 sample/s and the duration to 1800 seconds. **Note**: If the Polarimetry Settings menu is not open, click or tap .
   - LabQuest: On the Meter screen, tap Mode. Change the duration to 60 minutes. Tap OK. Then go to the Graph screen. Tap the y-axis and change it to "Optical Rotation (°)". Tap the x-axis and change it to "Time (min)".

9. Add 10 mL of 6.0 M HCl to 10 mL of the 30% sucrose solution into a beaker and quickly transfer the mixed solution into an empty polarimeter sample cell. Accurately measure and record the height of the liquid in the cell to the nearest 0.1 cm. **DANGER**: *Hydrochloric acid solution,* HCl: *Causes severe skin and eye burns and damage. Harmful if swallowed or inhaled. Do not eat or drink when using this product. Do not breathe mist, vapors, or spray. May be corrosive to metals*.

10. Collect kinetics data.
    a. Place your filled sample cell in the polarimeter.
    b. Start data collection. Data collection will end automatically.
    c. Store the data, if necessary:
       - Instrumental Analysis: Data are stored automatically. Continue with part d.
       - LabQuest: To store the data, tap the File Cabinet icon. Then, continue with part d.
    d. Empty the polarimeter cell and clean it.

11. To use the Curve Fit tool to determine the best fit, reaction order, and the rate constant, follow the appropriate steps. **Note**: You may want to add a calculated column to convert Angle of Rotation to Concentration using the information you gathered in Part I.
    - Instrumental Analysis: Click or tap Graph Tools, , and choose Apply Curve Fit.
    - LabQuest: Choose Curve Fit from the Analyze menu.

Part III Kinetics of sucrose with invertase enzyme

12. Accurately prepare 25 mL of a 15 mg/mL invertase solution.

13. Add 10 mL of the 30% sucrose solution to 10 mL of 15 mg/mL invertase into a beaker and quickly add the mixed solution into a clean polarimeter sample cell. Accurately measure and record the height of the liquid in the cell to the nearest 0.1 cm. **Caution**: *Treat all laboratory chemicals with caution. Prudent laboratory practices should be observed.*

14. Collect kinetics data.
    a. Place your filled sample cell in the polarimeter.
    b. Start data collection. Data collection will end automatically. If using LabQuest, store the run.

15. Use the Curve Fit tool to determine the best fit, reaction order, and the rate constant. **Note**: You may want to add a calculated column to convert Angle of Rotation to Concentration using the information you gathered in Part I.

DATA TABLES

Part I Specific rotation of sucrose

15% Sucrose Sample

	Run 1	Run 2	Run 3	Average
Sample height (cm)				
Angle of rotation, α (°)				

30% Sucrose Sample

	Run 1	Run 2	Run 3	Average
Sample height (cm)				
Angle of rotation, α (°)				

DATA ANALYSIS

Part I Specific rotation of sucrose

1. Using the average observed rotation for 15% sucrose, calculate its specific rotation.

2. Using the average observed rotation for 30% sucrose, calculate its specific rotation.

3. Compare the above values to the accepted literature value for the specific rotation of sucrose and calculate the percent difference.

Part II Kinetics of sucrose with acid

4. What is the order of the reaction between sucrose and HCl? Explain.

5. Determine the rate constant for this reaction with respect to sucrose.

Part III Kinetics of sucrose with invertase enzyme

6. What is the order of the reaction between sucrose and invertase? Explain.

7. Determine the rate constant for this reaction with respect to sucrose.