  Experiment 15

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.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | | | |
| Sucrose |  | Glucose | Fructose |
| [α] = 66.5° |  | [α] = 52.7° | [α] = –92.4° |

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

α = [α] ℓ c

where α is the observed optical rotation in units of degrees, [α] 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

LabQuest or computer interface

LabQuest App or Logger Pro

Vernier 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. Connect the Vernier Polarimeter cables to their respective ports on your Vernier interface. Start the data-collection program and choose New from the File menu.
4. Calibrate the Polarimeter.
   1. 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.
   2. Place the cell in the Polarimeter.
   3. Start data collection and slowly rotate the analyzer clockwise or counterclockwise until data collection stops (15 s). Note: If you are using a LabPro interface, only rotate the analyzer while data collection is active. Allow a few seconds at both the beginning and ending of data collection without moving the analyzer.

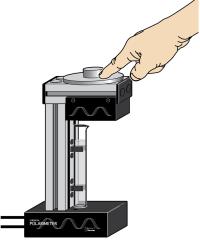


Figure 2  Rotation of the analyzer

1. Record the first angle above 0° where the illumination is at a maximum for the blank. There are several ways to locate this angle using analysis features in Logger Pro or LabQuest App. One way to locate this angle is to use a Gaussian fit.
2. Store the run. In Logger Pro, do this by choosing Store Latest Run from the Experiment menu. In LabQuest App, you can store a run by tapping the File Cabinet icon.
3. You are now ready to add the optically active sample into the Polarimeter cell.
   1. 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.
   2. Place the sample cell in the Polarimeter.
   3. Start data collection and slowly rotate the analyzer clockwise or counterclockwise until data collection stops.
4. Record the first angle above 0° where the illumination is at a maximum for the sample.
5. Repeat Steps 6–8 two more times for the 15% sucrose solution and a total of three times for the 30% sucrose solution. You can reuse your solutions in the following experiments.

Part II Kinetics of Sucrose with Acid

1. Prepare the Vernier Polarimeter for data collection as you did in Part I. Note: The reaction mixture you are preparing is time-sensitive; only prepare it when the Polarimeter is set up properly, calibrated, and ready to collect data.
2. Calibrate the Polarimeter.
   1. Add 20 mL of the 30% sucrose solution to the Polarimeter cell and insert the cell into the Polarimeter. Note: 20 mL is not 10 cm in the cell; make sure both your volumes and heights are consistent throughout the experiment to reduce errors.
   2. Start data collection and rotate the analyzer several times back and forth until data collection stops.
   3. Store the run.
   4. Record the first angle above 0° where the illumination is at a maximum. This is angle at time zero.
   5. Remove the cell and the solution.
3. 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.
4. Collect kinetics data.
   1. Place your filled sample cell in the Polarimeter.
   2. Start data collection and rotate the analyzer several times back and forth until data collection stops.
   3. Store the run.
   4. Continue to repeat this process of collecting data runs every two minutes for 60 minutes. If possible, take another reading at 120 minutes.
5. Generate a table in your lab notebook with columns for Time and Angle of Rotation.
   1. Record the time values in minutes in your lab notebook.
   2. Determine the first angle above 0° where the illumination is at a maximum. Record this value in your lab notebook. Remember to include your time zero data point from Step 11.
6. To analyze this time-dependent data, it is necessary to manually enter the data, plot it, and find its best fit.

Logger Pro (LabQuest App users, see below)

1. Choose Add Page from the Page menu. Select New Data Set and Graph. Click OK. In the data table of Page 2, you will see a new data set with an “X” column “Y” column.
2. To rename the X column, double click on the column heading. Enter Time as the column Name, Time as the Short Name, and min as the Units. Click Done.
3. In the same manner, name the Y column. Enter Angle of Rotation as the column name, Angle as the short name, and ° (the degree symbol) as the unit by choosing the symbol from the drop-down menu.
4. Enter your data in the appropriate columns. Make sure the graph is displaying Time on the x-axis and Angle of Rotation on the y-axis.
5. Continue to Step 16.

LabQuest App

1. Save your raw polarimetry data file on to LabQuest. Choose New from the File menu.
2. Tap the Table tab. You should see a data set with a column named “X” and a column named “Y.”
3. To rename the x column, tap the x-column heading; a new window will appear where you can name the column. Enter Time as the column name and min as the units. Tap OK.
4. In the same manner, rename the y column. Enter Angle as the column name and deg as the units. Tap OK.
5. Enter your data in the appropriate columns. Return to the Graph screen and confirm the graph is displaying Time on the x-axis and Angle on the y-axis.
6. In Logger Pro or LabQuest App, choose Curve Fit from the Analyze menu 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.

Part III  Kinetics of Sucrose with Invertase Enzyme

1. Accurately prepare 25 mL of a 15 mg/mL invertase solution.
2. Prepare the Vernier Polarimeter for data collection as you did in Part I. Note: The reaction mixture you are preparing is time-sensitive; only prepare it when the Polarimeter is set up properly, calibrated, and ready to collect data.
3. Calibrate the Polarimeter.
   1. Add 20 mL of the 30% sucrose solution to the Polarimeter cell and insert the cell into the Polarimeter. Note: 20 mL is not 10 cm in the cell; make sure both your volumes and heights are consistent throughout the experiment to reduce errors.
   2. Start data collection and rotate the analyzer several times back and forth until data collection stops.
   3. Store the run.
   4. Record the first angle above 0° where the illumination is at a maximum. This is the angle at time zero.
   5. Remove the cell and the solution.
4. 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 an empty 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.
5. Collect kinetics data.
   1. Place your filled sample cell in the Polarimeter.
   2. Start data collection and rotate the analyzer several times back and forth until data collection stops.
   3. Store the run.
   4. Continue to repeat this process of collecting data runs every two minutes for 60 minutes. If possible, take another reading at 120 minutes and 180 minutes.
6. Generate a table in your lab notebook with columns for Time and Angle of Rotation.
   1. Record the time values in minutes in your lab notebook.
   2. Determine the first angle above 0° where the illumination is at a maximum. Record this value in your lab notebook. Remember to include your time zero data point from Step 19.
7. To analyze this time-dependent data, it is necessary to manually enter the data, plot it, and find its best fit.

Logger Pro (LabQuest App users, see below)

1. Choose Add Page from the Page menu. Select New Data Set and Graph. Click OK. In the data table of the newly generated Page 2, you will see a new data set with a column named “X” and a column named “Y.”
2. To rename the X column, double click on the column heading. Enter Time as the column name, Time as the short name, and min as the units. Click Done.
3. In the same manner, name the Y column. Enter Angle of Rotation as the column name, Angle as the short name, and ° (the degree symbol) as the unit by choosing the symbol from the drop-down menu.
4. Enter your data in the appropriate columns. Make sure the graph is displaying Time on the x-axis and Angle of Rotation on the y-axis.
5. Continue to Step 24.

LabQuest App

1. Save your raw polarimetry data file on to your LabQuest. Choose New from the File menu.
2. Tap the Data Table tab. You should see a data set with a column named “X” and a column named “Y.”
3. To rename the x column, tap the x-column heading; a new window will appear where you can name the column. Enter Time as the column name and min as the units. Tap OK.
4. In the same manner, rename the y column. Enter Angle as the column name and deg as the units. Tap OK.
5. Enter your data in the appropriate columns. Return to the Graph screen and confirm the graph is displaying Time on the x-axis and Angle on the y-axis.
6. In Logger Pro or LabQuest App, choose Curve Fit from the Analyze menu 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

Angleblank (°) = \_\_\_\_\_\_\_\_\_\_

|  | Run 1 | Run 2 | Run 3 | Average |
| --- | --- | --- | --- | --- |
| Sample height (cm) |  |  |  |  |
| Anglesample (°) |  |  |  |  |
| Angle of rotation, α (°) = Anglesample – Angleblank |  |  |  |  |

30% Sucrose Sample

Angleblank (°) = \_\_\_\_\_\_\_\_\_\_

|  | Run 1 | Run 2 | Run 3 | Average |
| --- | --- | --- | --- | --- |
| Sample height (cm) |  |  |  |  |
| Anglesample (°) |  |  |  |  |
| Angle of rotation, α (°)  = Anglesample – Angleblank |  |  |  |  |

Note: Record Parts II and III in your laboratory notebook.

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

1. What is the order of the reaction between sucrose and HCl? Explain.
2. Determine the rate constant for this reaction with respect to sucrose.

Part III  Kinetics of Sucrose with Invertase Enzyme

1. What is the order of the reaction between sucrose and invertase? Explain.
2. Determine the rate constant for this reaction with respect to sucrose.