INVESTIGATING SUGAR STEREOCHEMISTRY WITH LEGO® POLARIMETRY
Objectives

- Understand the property of optical activity and its relation to stereochemistry
- Use a Lego® polarimeter to demonstrate the key components necessary to measure optical activity
- Apply polarimetry to determine the sugar concentration in unknown samples

Introduction

Optical Activity and Stereochemistry

Optics describes the interactions of light and matter.

Optics is a field which has long fascinated scientists. At the beginning of the nineteenth century, it was documented that plane polarised light exhibits very different behaviour when interacting with different substances. Some molecules rotated the plane of polarised light and were therefore noted to be optically active.

Glossary: Plane polarised light is light in which all electric fields oscillate in the same direction. That is, all light has the same orientation.

**Fig. 1. Plane Polarised Light Interacting with an Optically Active Sample**

Louis Pasteur investigated this behaviour, and, from his observations, concluded that it is molecular asymmetry (dissymétrie) which accounts for the optical activities of chemicals. This is the founding idea of stereochemistry. It was his investigation of tartaric acid crystals (growing in wine sediment) which led Louis Pasteur to his revolutionary conclusion that optical activity is dependent upon stereochemistry.
Stereochemistry is the study of chemical configurations, the 3D arrangement of atoms in space within a molecule.

**Configuration** refers to the 3D spatial arrangement of molecular substituents, not to be confused with connectivity or conformation! **Connectivity** refers to the bonding interactions between substituents and **conformation** refers to the rotational positions of atoms or groups about a single bond.

![Fig. 2. Distinguishing Connectivity, Conformation, and Configuration](image)

A and B are constitutional isomers with different connectivity.

A and C are conformers, interconverted with rotation.

A and D are stereoisomers with different configurations.

Importantly, an atom (usually carbon) is labelled a **stereocentre** if it has four inequivalent substituents. **Stereoisomers** cannot be interconverted by rotation.

This is a stereocentre, as the carbon has four inequivalent substituents: hydrogen, hydroxy, alkyl and alkenyl functionalities.

This is **NOT** a stereocentre as it has two methyl substituents.

**Glossary:** A substituent is an atom or group of atoms attached to a specified centre.
Substituents may be arranged around a stereocentre so as to create different enantiomers, which are a class of stereoisomers. Enantiomers have the notable property of chirality.

An object is described as chiral if its mirror images are non-superimposable.

**Figure 3. Illustrative Examples of Chirality**

Consider molecule A and its reflection in a mirror plane. In order to test if the molecule is chiral, we attempt to superimpose (overlay) mirror images A and B, as illustrated with the hands and flask above. First, we rotate A about its C-OH bond to put the I atom in the right position. However, if we now try to put rotated A on top of B, we see the CH₃ and H positions are switched! Hence, we cannot rotate A in such a way that it can be superimposed on its mirror image. Therefore, this compound is chiral, and the two reflections are enantiomers.

In lab question: Of A, B, and rotated A name the pair of:

a) Conformers
b) Stereoisomers

Extension: Make a model! Try using lolly sticks and blutack™ to model these spatial relationships with a 3D representation of molecule A?

Conversely, consider molecule C (replaced CH₃ with H). With the same test, we see we *can* overlay rotated and reflected B. This molecule is achiral.
It is chirality which is responsible for the optics of a molecule; chiral molecules rotate the plane of polarized light.

**Molecules that are optically active are chiral.**

In Lab Question: Can you now explain why Louis Pasteur observed that tartaric acid crystals rotate the plane of polarized light? (Hint: refer to the picture of the crystals pg. 2)

Enantiomers rotate the plane of polarized light in equal and opposite directions. In a **racemic** mixture, enantiomers are present in equal proportions, and so the sample is not optically active. Hence, the measurement of the angle of rotation is very informative. This is accomplished with a **polarimeter**.

**Figure 4. Racemic Mixture**

| 100% (R,R)-tartaric acid (left) specific optical rotation: +24.8 degrees |
| 100% (S,S)-tartaric acid (right) specific optical rotation: -24.8 degrees |

In lab question: Test for chirality to show that these tartaric acids are enantiomers (non-superimposable mirror images). What would be the optical rotation of a solution with 50% (R,R)-tartaric acid and 50% (S,S)-tartaric acid? What about 40% (R,R) and 60% (S,S)?

**Applications of Polarimetry**

Polarimetry finds an important application in the food industry. The observed optical rotation of a sample is dependent on its concentration. This should be distinguished from specific optical rotation, which is standardized to 1 g mL\(^{-1}\) concentration and a sample path length of 1 dm. The phenomenon of optical rotation provides an important analytical method for the determination of concentrations and proportions of sugars in a sample.

The specific optical rotation of glucose, sucrose, and fructose is well documented. Modern industry therefore applies these measurements to compositional analyses of sugar containing compounds such as honeys and syrups. In 2013, the EU promoted honey to the top ten list of foods most susceptible to food fraud because the honey industry is so lucrative that there is a large financial incentive to contaminate high quality honey with cheaper sugars. Measurements of optical rotation are one of many quantitative tests analytical chemists use to ensure quality standards are met.
More recently, polarimetry has also been investigated as a non-invasive way to monitor glucose levels for people with diabetes. From measurements of optical rotation of the anterior chamber of the eye, glucose concentrations in the blood may be calculated using the observed rotation’s relationship with concentration.

Extension: Can you research more ways polarimetry is useful in the modern world?

Polarimeter

A polarimeter measures optical activity.

A polarimeter measures optical activity and consists of four basic components. First it must contain a light source. The standard light source has the wavelength of the sodium D-line, 589 nm. The polarimeter must then have a polarizing film, which the light passes through; this ensures all light has the same orientation when it reaches the sample cuvette. Finally, an analyser film and detector are used to measure the angle of rotation.

Figure 5. Schematic of a Polarimeter

A. The Detector (in this polarimeter, the analyser is rotated until no light is observed)
B. The Analyser
C. The Optically Active sample (placed inside the machine to limit exposure to non-polarised light)
D. The Polarising Film
E. The Light Source (in this polarimeter, a sodium lamp)
Pre-Lab Exercise:
In this lab, you will use polarimetry to determine the concentration of sugar in an unknown solution.

Can you identify the stereocentres?

(Hint: There are nine! Refer to page 3).

You will construct a polarimeter from Lego® bricks. This polarimeter utilises a light source of an LED torch. The light then passes through a polarisable film, which ensures all light reaching the sample has a uniform orientation. There is a chamber for the sample tube, in which the light will be passed through test solutions. This polarimeter then uses a red LED to detect current. The observed optical rotation of the sample corresponds to the minimum observed voltage.

In Lab Question: On the following page is a schematic of the Lego® polarimeter you will construct. Referring to Figure 5, assign the following roles to the respective component:

Sample Tube Analysing Polarising Filter Polarizing Filter
Light Source LED Detector
Lego® Polarimeter

A. 
B. 
C. 
D. 
E.
Practical work

Health & Safety

The sugar solutions used in this practical are non-hazardous. Voltages used in polarimetry are low such that it is very unlikely to cause harm. Avoid shining LED torches in eyes. The LED detector used will not overheat. The multimeter should only be used in completing the circuit instructed.

Procedure

**Equipment Provided**
- Multimeter XL830L
- Black Electrical Lead
- Red Electrical Lead
- Red Crocodile Clip
- Black Crocodile Clip
- 9 LED Torch
- 2 Red LED 5 mm (one backup)
- Laminated Protractor
- Plastic dial/ dial polarising film (attached)
- Fixed Polarizing Film
- 2 Specimen Tubes*
- 5x50 mL volumetric flasks*
- Unknown Sugar/ Salt Mix
- 5x50 mL beakers
- Plastic Spoon
- Stirrer
- 2 Decimal Place Mass Balance
- Permanent Marker
- Lego® Set
- 6 Pipettes
- Wash Bottle
- Filter Funnel
- Lemonade

*Different sized volumetric flasks and specimen tubes can be used, according to availability. The key is to ensure the path length is as long as possible.

**Equipment Needed:**
- Deionised Water (Garage, £1.50/L)
- Ruler
- Table Sugar
- Calculator
- Kitchen towel

**Preparation: Constructing the Lego® Polarimeter**
First you will construct a Lego® polarimeter. Before beginning your build, you must test your LED light detector. You are provided with two red LED detectors.

1. Locate your multimeter. Attach the red wire to the red crocodile clip and the longer LED leg. This is the anode. Attach the black wire to the black crocodile clip and the short LED leg. This is the cathode. Note: You should separate the legs of the LED detector to prevent a short circuit!
2. Attach the red wire to the ‘V Ω mA’ port and the black wire to the ‘COM’ port.
3. Turn the dial anticlockwise to **200 m** (200 mV). You should observe a voltage with a non-zero value on the multimeter. If you do not, your LED sensor may be faulty, and you should test the replacement before proceeding.

Construct your polarimeter following the attached instructions. See tips before building!

**Tips: Construction of Lego® Polarimeter**
- The LED light may not fully fit into the technic brick. Simply insert the detector as far as possible but note too much pressure may cause the LED to break!
- Ensure the black and red wires are fully inserted into the multimeter in order to measure an accurate voltage.
- Refer to the picture of the final product above for the correct orientation of the polarimeter.
Part I: Determination of the Specific Rotation of Sugar

Determine the specific rotation of sugar according to:

\[ \alpha = \frac{\alpha}{c \cdot l} \]

Where \( \alpha \) is the specific rotation, \( \alpha \) is the observed rotation, \( c \) is the concentration in g mL\(^{-1}\) and \( l \) is length of the sample holder in dm.

To determine this value, you will measure the observed rotation of four standard solutions with known sugar concentrations of 0.0 g mL\(^{-1}\), 0.1 g mL\(^{-1}\), 0.4 g mL\(^{-1}\), and 0.6 g mL\(^{-1}\). You will then exploit the linear relationship between observed rotation and concentration to determine a value of specific rotation for this polarimeter. This is a calibration step.

**Glossary:** Calibration is the process of comparing a measured quantity to a literature value to verify accuracy of future results.

Make a Standard Solution:

1. Calculate the number of grams of sugar you will need to make a standard solution of the desired concentration:

<table>
<thead>
<tr>
<th>Desired Concentration (g mL(^{-1}))</th>
<th>Mass of Sugar (g) in 50 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td></td>
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<tr>
<td>0.1</td>
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<tr>
<td>0.4</td>
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<tr>
<td>0.6</td>
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</tbody>
</table>

2. Place a 100 mL beaker on the balance before pushing ‘tare’, which will reset the denoted mass of the beaker to be 0. Weigh out the desired mass of sugar into the beaker.
3. Spray a small amount of deionised water into the beaker, using the stirrer to help the sugar dissolve.

**Tip:** Beware not to surpass the 50 mL line when dissolving sugar in the beaker, as this is the total volume of the stock solution! Use the minimum solvent required to dissolve the sugar. Start with approximately 10–15 mL and use a stirrer to help solvation.

4. Use the funnel to transfer your mixture to a 50 mL volumetric flask. Wash the beaker with deionised water and transfer to the flask, to ensure all measured sugar goes into the standard solution. Again, beware NOT to surpass the dilution mark on the flask. If you do, the concentration will be lower than required and you will need to start again.
5. Dilute to the mark on the volumetric flask, ensuring the bottom of meniscus is in line with the mark. If solutions have bubbles, lightly tap the flask to remove.
6. Stopper the volumetric flask and invert 10 times. Note with the higher concentrations of sugar, the solutions may appear opaque. Attempt to ensure all sugar is dissolved. You may need to warm the solution by placing the stoppered flask into a beaker of warm water.
7. Use the permanent marker to label the standard solution with its concentration. Permanent marker can be removed from glass with an alcohol wipe.
8. Repeat steps 2–7 to produce four stock solutions; one of just solvent and three standards of known concentration.

Calibrating the Polarimeter:

Tip: Measurements using a polarimeter are most accurate when performed in a dimly lit room. This minimises the possibility of non-polarised light reaching the LED detector. Try to keep ambient light to a minimum by placing the polarimeter in the box (as on page 10) and flipping down the lid before recording each measurement from the multimeter. If possible, work in a darkened room.

1. Use the ruler to measure 8 cm (0.8 dm) from the bottom of the sample tube. Use the permanent marker to mark this point. This gives you a consistent path length of 0.8 dm for following calculations.
2. Use a bulb pipette to transfer solution to the sample tube, filling to the 0.8 dm line.
3. Insert the sample tube into the polarimeter. Ensure the tube is uniformly covering the window to the LED sensor.

In Lab Question: Why is it important to cover as completely as possible the window to the detector with the sample tube?

4. Rotate the pointer to -20°. Note: if the pointer rotates, so does the polarising film.
5. Flip down the lid of the box to block out contaminating light and record the value on the multimeter (set again to 200 m) once it has settled to a consistent voltage. As this instrument is very sensitive, this value may continue fluctuating. Spend a maximum of 1 minute on each measurement.
6. Use the chart below to record measurements for rotations -20° to +20°. This is the rough measurement of the optical activity of the sample.
7. Then, record at least three additional readings to pinpoint the minimum voltage. This voltage corresponds to the observed optical rotation of the sample, as it is the value at which the least amount of light reaches the LED detector.
8. Repeat steps 2–7 for all three standard solutions and the deionised water. Between each trial, be sure to use a clean bulb pipette to avoid contamination of stock solutions. Rinse the sample tube with deionised water and attempt to dry as much as possible with a piece of kitchen towel between measuring each standard. This is good lab practice as extra deionised water in the sample tube could dilute your known concentration.
Rough Measurement of rotation of polarising film:

<table>
<thead>
<tr>
<th>Film rotation (degrees)</th>
<th>0.0 g/mL Voltage (mV)</th>
<th>0.1 g/mL Voltage (mV)</th>
<th>0.4 g/mL Voltage (mV)</th>
<th>0.6 g/mL Voltage (mV)</th>
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</thead>
<tbody>
<tr>
<td>-20</td>
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<td>-15</td>
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<td>+20</td>
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</table>

Additional Measurement to Pinpoint Minimum Voltage:

<table>
<thead>
<tr>
<th>0.00 g/mL</th>
<th>0.10 g/mL</th>
<th>0.40 g/mL</th>
<th>0.60 g/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotation (degrees)</td>
<td>Voltage (mV)</td>
<td>Rotation (degrees)</td>
<td>Voltage (mV)</td>
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**Tip:** For more accurate results, record additional measurements to confirm the angle of minimum voltage. You may also wish to confirm this minimum with a graph!

9. Record the protractor angle corresponding to the minimum voltage for each stock solution and the solvent solution in the table on the following page. If you measure a non-zero value for the solvent, you should subtract this value from the measured protractor rotation for each stock solution, as we only wish to measure the optical activity of sucrose. The net angle of film rotation corresponds to your observed optical rotation, \( \alpha \).
In Lab Question: Why do we expect the solvent, water, to have an optical rotation of zero?

**Determination of Observed Rotation:**

<table>
<thead>
<tr>
<th>Concentration of Sugar (g mL(^{-1}))</th>
<th>Optical Rotation (degrees) at Minimum Voltage</th>
<th>Observed Rotation (degrees) Relative to Solvent: (\alpha) (Optical Rotation– Solvent Rotation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td></td>
<td></td>
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<tr>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.60</td>
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<td></td>
</tr>
</tbody>
</table>

10. Thus, given observed rotation, concentration, and pathlength, determine \([\alpha]\), the specific rotation of sugar, for each concentration. This value should be the same for each concentration (allowing for experimental error).

Recall:

\[
[\alpha] = \frac{\alpha}{c \ l}
\]

**Determination of Specific Rotation of Sugar:**

<table>
<thead>
<tr>
<th>Concentration (g mL(^{-1}))</th>
<th>Specific Rotation (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td><strong>Average Specific Rotation</strong></td>
<td></td>
</tr>
</tbody>
</table>
Part II: Determining the Concentration of an Unknown Sample of Sugar

In Lab Question: Why is it possible for us to use polarimetry to work out the relative proportions of sugar and salt in a mixture? (Hint: Refer to the Introduction!)

Determining the Concentration of Sugar in a Sugar and Salt Mixture:
1. Shake the unknown sugar and salt mixture within the sample tube to ensure it is properly mixed and any lumps are dissipated.
2. Referring to the procedure for making standard solutions in Part I, make a solution of approximately 10.00 g of the unknown mixture in 50 mL of water. Determine this concentration.

<table>
<thead>
<tr>
<th>Mass of Sample Mixture (g)</th>
<th>Concentration of Unknown Solution (g mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

3. Use a clean bulb pipette to transfer the solution to the sample tube, filling to the 0.8 dm line.
4. Use the same method as outlined in Part I to determine the minimum observed voltage for the sugar and salt mixture. First complete a rough survey to locate the range of the minimum, then pinpoint the minimum with additional measurements.

Rough Measurement of Minimum Voltage:

<table>
<thead>
<tr>
<th>Film rotation (degrees)</th>
<th>Unknown Sugar and Salt Mixture Voltage (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20</td>
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<tr>
<td>-15</td>
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<td>-10</td>
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<td>+15</td>
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<td>+20</td>
<td></td>
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</tbody>
</table>
Additional Measurement to Pinpoint Minimum Voltage:

<table>
<thead>
<tr>
<th>Unknown Sugar and Salt Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotation (degrees)</td>
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<tr>
<td>---------------------</td>
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</table>

5. Use the table below to find the proportion of sugar in the sugar and salt mixture. Consider the following strategy:

Once the observed rotation of the sugar, $\alpha$, is known, return to the formula used in Part I and use your determined specific rotation [$\alpha$] to find the concentration of sugar in the standard solution. Remember, if the solvent optical rotation is nonzero we must account for this contribution to the optical activity. Then, deduce the number of grams of sugar in the solution, with known volume of 50 mL. This will allow you to calculate the grams of salt (knowing the initial mass of the unknown sample) and thus the proportion of sugar to salt in the sample.

Determination of the Proportion of Sugar and Salt in Unknown Solution:

<table>
<thead>
<tr>
<th>Optical Rotation of Sugar (degrees) at Minimum Voltage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed Rotation Relative to Solvent (degrees): $\alpha$ (Recorded Rotation–Solvent Rotation)</td>
<td></td>
</tr>
<tr>
<td>Concentration of Sugar in the Sample (g mL$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>Mass of Sugar in 50 mL (g)</td>
<td></td>
</tr>
<tr>
<td>Mass of Salt in 50 mL (g)</td>
<td></td>
</tr>
<tr>
<td>Proportion of Sugar to Salt in the Unknown</td>
<td></td>
</tr>
</tbody>
</table>
Part III: Determining the concentration of Sugar in Lemonade

1. Use a clean bulb pipette to transfer the lemonade into a cleaned sample tube. Fill to the 0.8 dm line.
2. Repeat the procedure from Part II to measure the observed optical rotation of the sample.

Rough Measurement of Minimum Voltage:

<table>
<thead>
<tr>
<th>Film rotation (degrees)</th>
<th>Lemonade Voltage (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20</td>
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<td>-15</td>
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<td>+15</td>
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<td>+20</td>
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</table>

Additional Measurement to Pinpoint Minimum Voltage:

<table>
<thead>
<tr>
<th>Lemonade</th>
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</thead>
<tbody>
<tr>
<td>Rotation (degrees)</td>
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<td>-------------------</td>
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</tbody>
</table>
3. Determine the concentration of sugar in lemonade from the observed optical rotation, \( \alpha \), and the specific optical rotation from Part II, \([\alpha]\). Refer again to the equation in Part I. Remember to subtract the rotation of solvent, if nonzero.

**Determination of the Concentration of Sugar in Lemonade:**

<table>
<thead>
<tr>
<th>Observed Rotation (degrees) of Lemonade at Minimum Voltage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed Rotation Relative to Solvent (degrees): ( \alpha ) (Recorded Rotation minus Solvent Rotation)</td>
<td></td>
</tr>
<tr>
<td>Concentration of Sugar in the Lemonade (g mL(^{-1}))</td>
<td></td>
</tr>
</tbody>
</table>
Post Lab:

1. The literature value of the specific rotation of sucrose is +66.5°. Propose reasons for any differences between your determined value of [α] and the literature value. Why was it necessary to calibrate the polarimeter you made and use this value of specific rotation in subsequent calculations instead of using the literature value?

2. The unknown substance has 11 grams of salt for every 27 grams of sugar. How does your calculated result compare to the known value? What factors might account for any differences?

3. Consider the listed nutrition information on the can of lemonade. How does your calculated result compare to the known value? What factors might account for any differences?

4. Comment on the accuracy of the Lego® polarimeter. What are some potential sources of error? How can the accuracy of this polarimeter be improved? (Hint: Consider your answers for questions 1-3).

Invert sugar is made from the hydrolysis of sucrose, which produces glucose and fructose.

\[
\text{Sucrose} \xrightarrow{\text{H}_2\text{O}, \text{H}^+} \text{Glucose} + \text{Fructose}
\]

1. Invert sugar has an optical rotation of -39°. Suggest a reason as to why its name is “invert” sugar.

One way to monitor the conversion of sucrose to invert sugar is by polarimetry. As sucrose is converted into invert sugar, we expect to observe an optical rotation which is initially dextrorotatory (rotates the plane of polarised light right, +) then becomes laevorotatory (rotates the plane of polarized light left, -).

2. Noting that observed optical rotation is proportional to the concentrations of optically active compounds within the mixture, how can we use the data to track the progress of the reaction?

Extension:

1. Suggest a mechanism for the conversion of sucrose to invert sugar.

2. Why is water a good solvent in which to measure optical rotation? Hint: refer to your data for the solvent solution optical rotation.
References consulted:


Image credits


p.2. Khan Academy


Use of ChemDraw and Chemix to create diagrams
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