Experiment 6

The Rotation of Polarized Light by Chiral Molecules

Smell of (*S*)-limonene   Smell of (*R*)-limonene

The Task

In this experiment you will learn how a polarimeter works and use one to measure the optical activity of limonene, sucrose, glucose, fructose and the hydrolysis of sucrose.

Skills

At the end of the laboratory session you should be able to:
- understand how a polarimeter works,
- make molecular models,
- use a volumetric flask to make up solutions of a known concentration.

Other Outcomes

- You will learn how to identify the chiral carbon atoms in a molecular model or structure.
- You will measure the optical rotation of a solution.
- You will learn to use a polarimeter.

The Assessment

You will be assessed on your ability to make up a series of solutions of different concentrations. See Skill 4.5. Make sure all your solutions are homogeneous by stirring them well.

Introduction

See Skill 13 for an explanation on the use of stick structures to describe the molecular structure of organic compounds. See how the stick representation in the structures below is much less cluttered and easier to follow. Understanding this notation is essential for this experiment.

The properties of a molecule are not completely determined by their elemental composition. Take, for example, the molecular formula C_3H_8O. This formula is consistent with three different molecules: *i.e.* 1-propanol, 2-propanol and methoxyethane.

| 1-propanol | 2-propanol | methoxyethane |
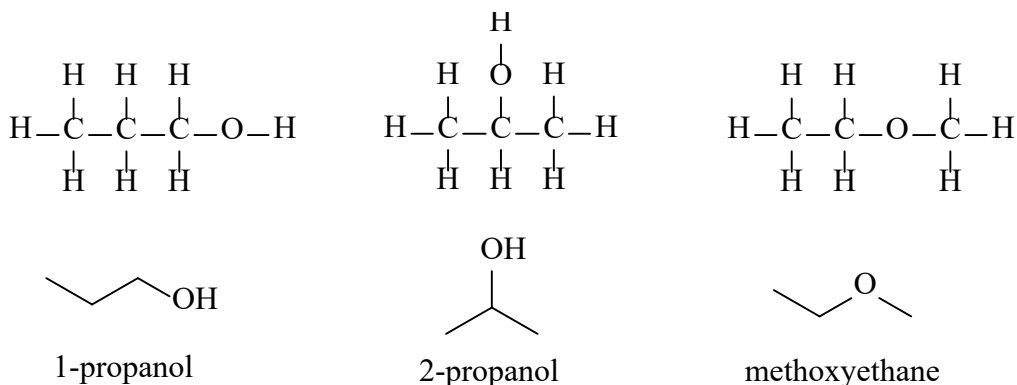
Molecules that have the same elemental composition are called *isomers*. Isomers that have different bonding structures, such as in the example above are called *constitutional* or *structural* isomers.

However, it is possible to have isomers that have exactly the same bonding structure, but which differ from one another in how the atoms are organised in space. These are called *stereoisomers*. Consider the (*E*) and (*Z*) isomers[1] of 2-butene shown below.
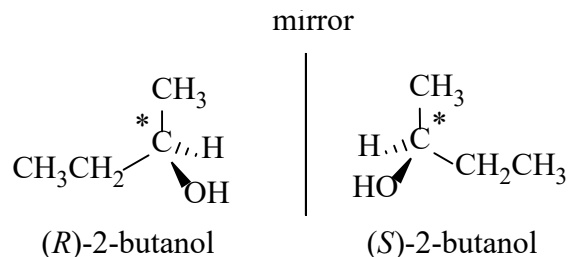

(*E*)-2-butene   (*Z*)-2-butene
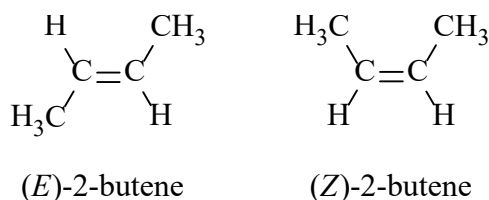
Both molecules consist of a CH_3 group single-bonded to a CH which in turn is double-bonded to another CH which is in turn single bonded to another CH_3 group, so the **order** of atoms and bonding is the same in both molecules. However, as the double bond locks each molecule into a rigid flat shape, the relative positions of the atoms in space are different - the CH_3 groups are on opposite sides of the double bond in the (*E*)-isomer and on the same side of the double bond in the (*Z*)-isomer. Such compounds are called *diastereoisomers*. They have different physical properties (*e.g.* melting and boiling points) and different chemical properties (*e.g.* react at different rates or give different products with the same reagents).

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[1] "E" comes from the German "entgegen" meaning opposite. "Z" comes from the German "zusammen" meaning together.

A special class of stereoisomers, called *enantiomers*, are mirror image pairs that cannot be superimposed on each other. These stereoisomers are called *enantiomers*. An example of a pair of enantiomers is provided by 2-butanol.

mirror

$$CH_3CH_2 \overset{\overset{\displaystyle CH_3}{\overset{*}{|}}}{\underset{OH}{C\cdots H}} \qquad \overset{\overset{\displaystyle CH_3}{\underset{*}{|}}}{\underset{HO}{H\cdots C}} CH_2CH_3$$

(*R*)-2-butanol   (*S*)-2-butanol

Molecules that have two enantiomeric forms are called *chiral*[2] and they generally contain at least one atom that is bonded to four different groups. This atom is called a *stereogenic centre*. The carbon atom marked with an asterisk in 2-butanol above is a stereogenic centre - it is bonded to four different groups, *viz.* CH_3, CH_2CH_3, OH and H.

Unlike diastereoisomers, enantiomers have identical chemical properties except when interacting with other chiral molecules. They also have identical physical properties except when interacting with polarized light. Why bother with distinguishing such molecules when they are identical in so many ways? The reason is that Nature is chiral - many of the molecules in living things (*e.g.* proteins and sugars) can form enantiomers, but only one of the possible enantiomers is actually found. This very particular selection of enantiomers in biological systems means that *all* enantiomers are distinguishable when introduced into a living cell. A classic example is that of thalidomide, a drug prescribed from 1957 to 1962 as a sedative and painkiller and as a treatment for morning sickness during pregnancy. Thalidomide is a chiral molecule and it was sold as a 50:50 mixture of both enantiomers. It was subsequently discovered that the (*R*)-enantiomer was effective against morning sickness, but the (*S*)-enantiomer was responsible for horrific birth defects. The tragedy of thalidomide stresses the importance of being able to distinguish enantiomers. On the bright side, the bitter experience gained with thalidomide has led to much more stringent testing of drugs before they enter the market.

The physical similarity between enantiomers makes separating them very difficult. Synthetic chiral molecules are, as a result, generally expensive to produce from non-chiral starting materials. As a living organism will only produce one of the possible enantiomers, natural products remain the most important source of chiral molecules. In some cases, such as the terpenes limonene and carvone, both enantiomers can be obtained from natural sources, although from different species. In the case of limonene, one enantiomer, (*R*)-limonene, is extracted from citrus rind while the other enantiomer, (*S*)-limonene, is obtained from pine resin.

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[2] "Chiral" comes from the Greek "cheir" meaning hand.

Optical Activity

Visible light consists of electromagnetic waves that oscillate in all possible planes perpendicular to the direction in which the light is travelling. Certain filters (*e.g.* calcite and Polaroid film) permit only light waves that are vibrating in one particular plane to be transmitted. After passage through such a filter, the light is called *plane-polarized light*. Jean-Baptiste Biot, a French scientist, discovered in 1815 that the plane of polarization was rotated by passing plane-polarized light through solutions of certain organic compounds. Molecules that possess this property are said to be *optically active*. Optical activity is a property of each individual molecule - the more molecules encountered by the light, the greater the amount of rotation. Consequently, the degree of rotation is dependent on the molecule, its concentration and the sample path length.

Measurement of optical rotation is performed using an instrument called a *polarimeter*. Here is how it works. Plane-polarized light is first obtained by passing light through a polarizer. If this polarized light is passed through a second filter (analyser) oriented parallel to the first, the maximum amount of light reaches our eye. If the orientation of the analyser is rotated, the amount of light being transmitted declines (*i.e.* the light dims). The polarimeter initially has the analyser oriented perpendicularly to the polarizer so that the transmission of light is at a minimum. When an optically active (chiral) material is placed between the polariser and analyser, the polarization vector of the light is rotated as described above, and the amount of light able to pass through the analyser increases, *i.e.* it will appear brighter. By rotating the analyser, the minimum transmission can be restored, and the angle through which the analyser must be rotated is a measure of how much rotation was produced by the sample solution (see Figure 1).
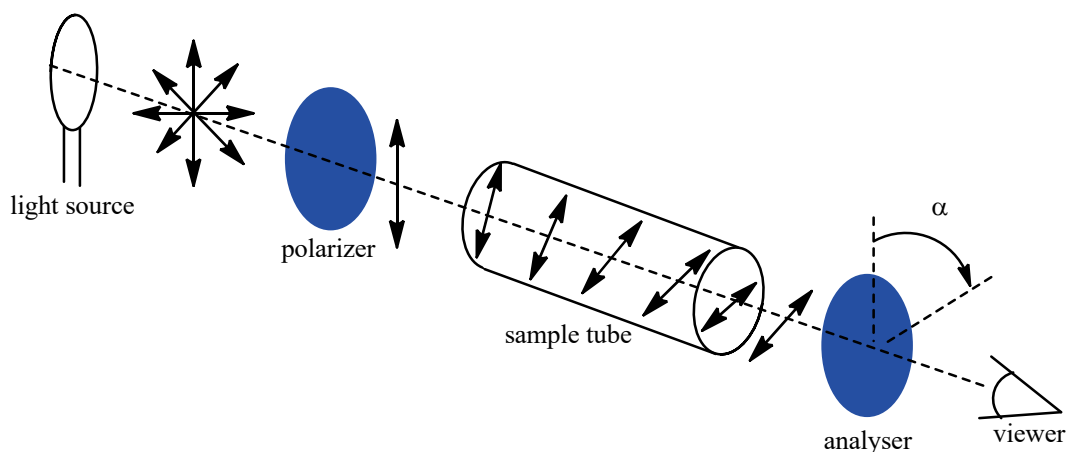

Figure 1: A diagram of a polarimeter.

Optically active compounds that rotate plane-polarized light to the right are given the symbol (+) and, conversely, compounds that rotate plane-polarized light to the left are given the symbol (–). A pair of enantiomers will rotate plane-polarized light **by the same amount**, but in opposite directions. The amount and direction of rotation of polarized light is a physical property of each and every compound and needs to be determined experimentally.

If you take a 50:50 mixture of two enantiomers, the (+) rotation from one enantiomer will exactly equal the (–) rotation from the other enantiomer. Such mixtures are called *racemic mixtures* or *racemates* and are denoted by the symbol (±). Racemic mixtures display an optical rotation of zero.

Molecular Model Building

Model kits are provided for this experiment. In your model kit the black balls represent carbon atoms; red balls oxygen atoms; white balls hydrogen atoms; blue balls nitrogen atoms; green balls chlorine atoms; and brown balls bromine atoms. The short grey bonds are for single bonds between all atoms except hydrogen. The long grey flexible bonds are for double and triple bonds. The short white bonds are used for bonding hydrogen atoms to the structure.

Safety

Chemical Hazard Identification
(S)-limonene – Flammable. Low to moderate toxicity. Do not inhale vapours.
(R)-limonene – Flammable. Low to moderate toxicity. Do not inhale vapours.
sucrose – non-hazardous
glucose – non-hazardous
fructose – non-hazardous
1 M HCl – non-hazardous

Risk Assessment and Control

Low risk.

Take care with the polarimeter cells.

Waste Disposal

Return your limonene samples to where you collected them from. **DO NOT** dispose of the limonene samples. All other solutions may be poured down the sink.

Experimental

> ## *This experiment is to be carried out in pairs.*

Part A   Molecular Models

(A1)   Use the molecular model kit to build a model of CH_2ClBr.

(A2)   Now build its mirror image.

For your logbook.

Are these molecules superimposable on each other?

(A3)   Use the molecular model kit to build a model of 2-butanol,

$$CH_3 - \underset{\underset{H}{|}}{\overset{\overset{OH}{|}}{C}} - CH_2CH_3 \quad .$$

(A4)   Now build its mirror image.

For your logbook.

Are these molecules superimposable on each other?

What name is given to these compounds to describe their stereochemical relationship?

Sketch the 3-dimensional structure of your models of 2-butanol and identify the stereogenic carbon centre.

Limonene belongs to a class of molecules called terpenes. Terpenes and their derivatives provide important biosynthetic building blocks and are major components of 'essential oils'. They are derived from *isoprene* units that are linked together in a "head-to-tail" arrangement. Limonene is a cyclic monoterpene associated with citrus smells.
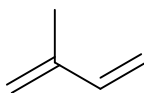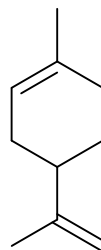
isoprene   limonene

(A5)   Sketch the structure of limonene in your logbook and indicate the stereogenic carbon centre.

(A6)   Using the model kit, build the two enantiomers of limonene. Check to see that your two models really are different structures.

Part B   Observing Optical Rotation

In this part of the experiment you are going to observe optical rotation using a simple polarimeter that you will construct yourselves.

The equipment required for building a polarimeter:
- 1 retort stand
- 2 rings
- 1 clamp
- 2 polarizing plates (actually lenses from Polaroid sunglasses)
- 2 cells (very large test-tube or measuring cylinder) each containing a different optical isomer of limonene
- Light and leads
- Box containing a light globe
- 1 powerpack per bench

We will follow the method shown in Figure 1. To get a feel for how the polarizers work, hold them up towards the window, one behind another, so that light is passing through both. Look through the polarizers and rotate one relative to the other. You should see that they alternate between light and dark as you change their orientations.   The polarisation direction of light that a polariser allows to pass is termed its *optical axis*. When the optical axes of both polarisers are parallel, light will pass through both. When the optical axes are perpendicular to one another no light should pass through, and you should just see black. See if you can locate the darkest possible orientation of the two polarizers, *i.e.* with their optical axes as close as possible to perpendicular.
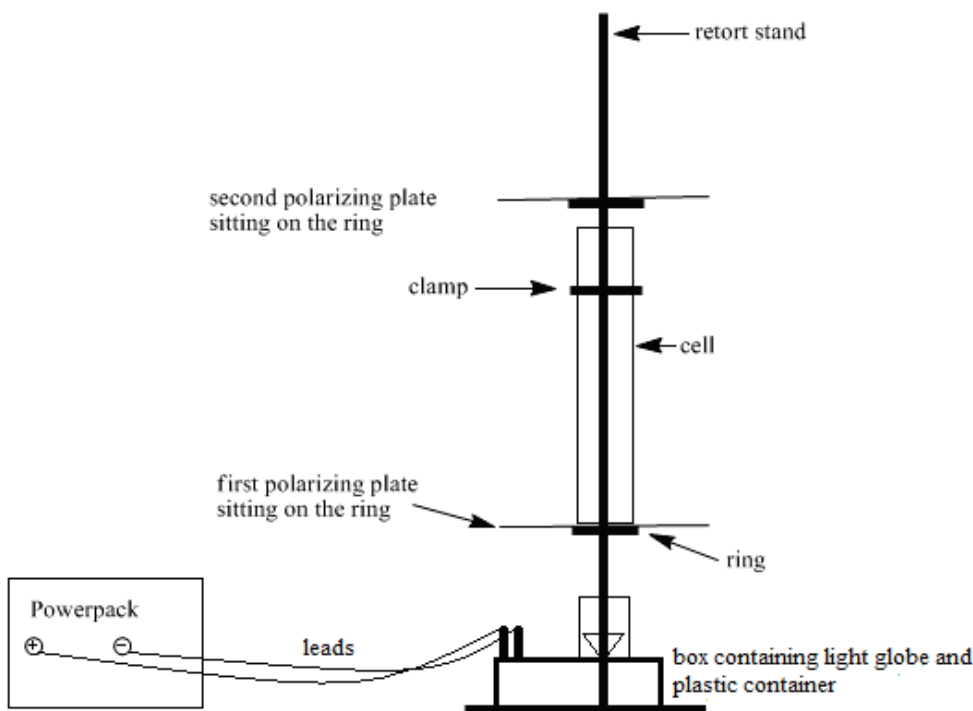
Figure 2: Schematic diagram of a polarimeter.

(B1) Place the box containing the light globe at the bottom of the retort stand, as shown in Figure 2. Connect the leads from the powerpack to the terminals on the box and switch on the light.

(B2) Clamp rings onto the retort stand to support the two polarizers. Place one polarizer on the bottom ring and one on the top ring. Allow space between the two polarizers for the cell containing the optically active solution (see Figure 2).

(B3) Look down from above the top polarizer towards the light and rotate the top polarizer until the optical axes of both polarizers are perpendicular, *i.e.* the blackest position.

(B4) Now clamp a cell containing one of the optical isomers of limonene between the two polarizers. Be careful not to change the orientation of the polarizers.

(B5) Rotate the upper polarizer to re-find the position of maximum darkness. Thus prove to yourself that that the solution has indeed rotated the polarization of the light.

(B6) Remove the cell, reorient the top polarizer to the blackest position and repeat steps (B4)-(B5) for the cell containing the other optical isomer of limonene.

For your logbook:
From measurements of individual samples of limonene, it is not possible to determine which is (+) and which is (–). Why do you think this is the case?

Part C   Measuring the magnitude of the optical rotation of limonene

To measure the absolute magnitude of the angle of rotation of polarized light by the two enantiomers of limonene we will use a modern commercial polarimeter.

(C1) Take a labelled polarimeter cell (either 5 or 10 cm pathlength) already filled with a solution of one of the enantiomers of limonene.

(C2) Measure the angle of its optical rotation using the polarimeter. (See the instructions provided with the instrument.) **Record the angle in your logbook.**

(C3) Find a labelled polarimeter cell filled with a solution of the other enantiomer and measure its angle of rotation. **Record the angle in your logbook.**

(C4) Measure angles of optical rotation of both enantiomers again, but using the other pathlength.

For your logbook

Identify which sample of limonene is (+) and which is (–).

Your demonstrator will now assign you different tasks. Some students will do Part D, others will do Part E. Share your results during the group discussion.

Instruction for Chemical Polarimeter

1. Connect the two Vernier Polarimeter cables to their respective ports on your Vernier LabQuest Mini. The graph on the monitor should have illumination on the y-axis and angle of the x-axis. If this is not the case, make sure the Polarimeter's analyzer is plugged into DIG1 on the LabQuest Mini and select New from the File menu.

2. Calibrate the Polarimeter:
   a- Pour blank solution (Ethanol for Limonene, and distilled water for sugars), in one of the cells (already provided for Limonene) to a height of 10cm. It is important to read the height to the nearest 0.1cm. Read to the bottom of the meniscus.
   b- Place the cell in the Polarimeter's cell holder. Ensure the light source under the cell is on.
   c- Start data collection with taping on the space bar on laptop and then slowly rotating the analyzer clockwise or counterclockwise as shown in the picture 3, until data collection stops (about 15 seconds). Slowly rotating analyzer produces smoother curves. Allow a few seconds at both the beginning and ending of data collection without moving the analyzer.
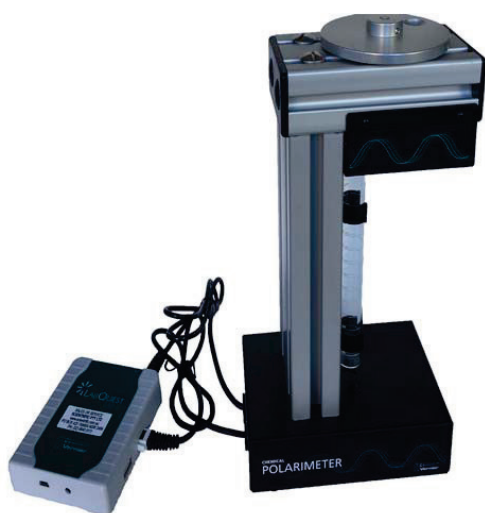
Picture 1: Vernier Polarimeter   Picture 2: Vernier LabQuest Mini
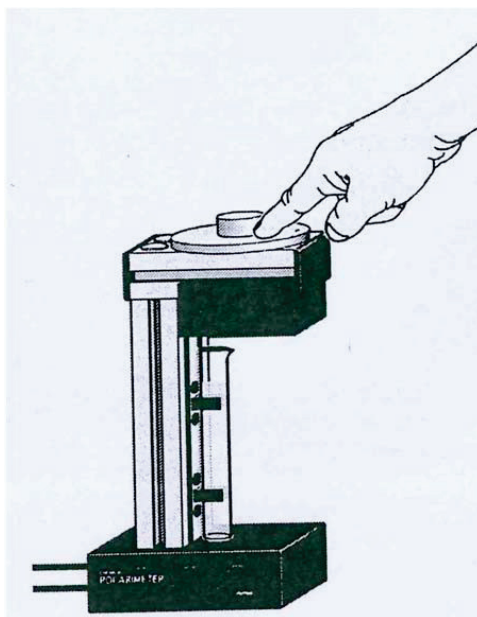
Picture 3: Rotation of the Analyzer

3. To save the graph, in Experiment from menu bar, choose 'Store Latest Run'. As a default, the software saves the latest run as Run1, to change the name, double click on the name on the left side column and change the name.

4. Record the first angle above 0° where the illumination is at a maximum for the blank. To do so, highlight the peak of the graph (Picture 4). In the menu go to Analyzer and choose ' Curve fit…'.
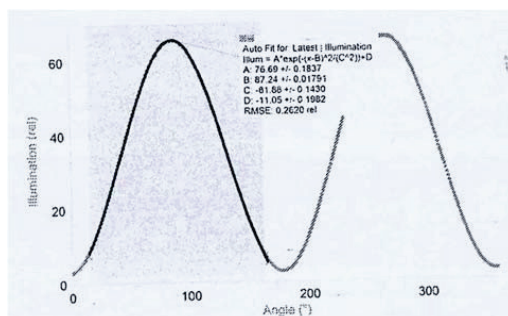
Picture 4: Selection for fits

5. From drop down list, choose Gaussian. Select Try fit, the fit will run automatically. The B Coefficient presented, **represents <u>the angle</u> at maximum illumination**. Click OK. Now the polarimeter is calibrated and ready to collect data from your sample:

6. Pour your sample in the Polarimeter cell to a height of 10cm/5cm, it is important to read the height to the nearest 0.1cm.

7. Place the sample cell in the Polarimeter's cell holder.

8. When you're ready, tap the space bar to start collecting the data. Slowly rotate the analyzer clockwise or counterclockwise until data collection stops.

9. To save and name the latest run follow step 3.

10. To find the angle for your blank follow step 4 and 5.

Calculations:

To determine the observed angle of rotation for the optically active sample (α):

1. Subtract the blank's angle of maximum illumination from the angle of maximum illumination for the sample.
2. A compound will consistently have the same specific rotation under identical experimental conditions. To determine the specific rotation of the sample, use Biot's law:

$$\alpha = [\alpha]lc$$

where α is the observed optical rotation in units of degrees, [α] is the specific rotation in units of degrees, l is the length of the cell in units of dm, and c is the sample concentration in units of grams per mL.

Part D Measuring the optical activity of sucrose, glucose and fructose

The term *sugar* encompasses a large group of carbohydrates such as glucose, fructose and sucrose. Common table sugar is sucrose, a disaccharide formed from the monsaccharides fructose and glucose.

glucose   fructose   sucrose
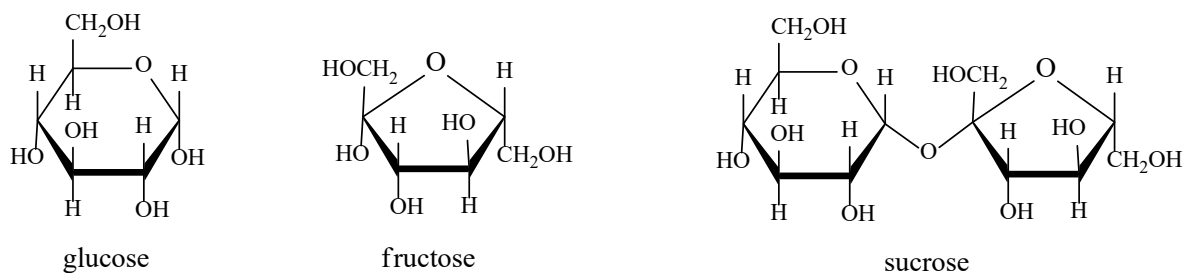
(D1) Sketch the structures of sucrose, glucose and fructose into your logbook, taking care to keep the spatial arrangement of atoms the same as in the above figures. For each molecule, identify the stereogenic carbon centre(s).

(D2) You will be assigned one of these sugars by your Demonstrator. Make up a 100 mL of a 20 % w/v aqueous solution (Skill 4.5) by weighing 20 g of your sugar on the top-loading balance (Skill 3.1B), transferring it to a 250 mL conical flask and adding 100 mL of water from a measuring cylinder.

(D3) Prepare 40 mL solutions of 15 %, 10 % and 5 % w/v by diluting your 20 % w/v stock sugar solution (Skill 4.5). Using measuring cylinders will give sufficient accuracy.

(D4) Measure the optical rotation of all 4 solutions in the polarimeter.

(D5) Repeat (D4). Make sure that you have waited at least 30 minutes between the first and second readings for each concentration
.
(D6) **Record your results in your logbook and on the white board for comparison and use by other pairs of students.**

For your logbook:

Draw a graph of the measured rotation versus the concentration of sugar and include it in your logbook.

Did the optical rotation of your sugar change with time? If so, you will need to plot 2 sets of points.

Part E   Monitoring the hydrolysis of sucrose by optical activity

The hydrolysis of sucrose results in the production of the two constituent monosaccharides, glucose and fructose. Since the optical activity of the reactants and products are different, we can follow this reaction using polarimetry.

(E1)   Weigh 20 g of sucrose in a 250 mL beaker (Skill 3.1B). Add 100 mL of 1.0 M HCl and heat gently on a hotplate for a few minutes. Stop heating if the solution starts to change colour.

(E2)   Allow the solution to cool and measure the optical rotation using the polarimeter. **Record the value in your logbook**.

(E3)   Separately, weigh out 10 g of fructose and 10 g of glucose (Skill 3.1B) and then mix them together in a 250 mL beaker. Add 100 mL of deionised water and measure the optical rotation of the solution using the polarimeter. **Record the value in your logbook**.

(E4)   The polarimeter cells that have contained the sugar solutions need to be cleaned before you return them to your Demonstrator. Rinse them all thoroughly a few times with tap water and then once with deionised water.

Group Discussion

Discuss your answers to the following questions. The idea is to make use of, where possible, your own and other groups' observations in this experiment.

1) What would you expect to observe in terms of the angle of rotation if you were to double the concentration of your sample while keeping the path length fixed? What about doubling the path length while keeping the concentration fixed?

2) Given your answer to (1), can you work out a formula to obtain the concentration of, for example, fructose in a solution using the measured angle of rotation and the path length?

3) Do you think that the hydrolysis of sucrose produced only single enantiomers of glucose and fructose or racemic mixtures of these monosaccharides?

4) Based on your identification of the stereogenic carbon centres in sucrose, how many different enantiomers of sucrose do you think there are?

References

James E. Garvin, *J.Chem.Ed*. **37**(10), 515 (1960).