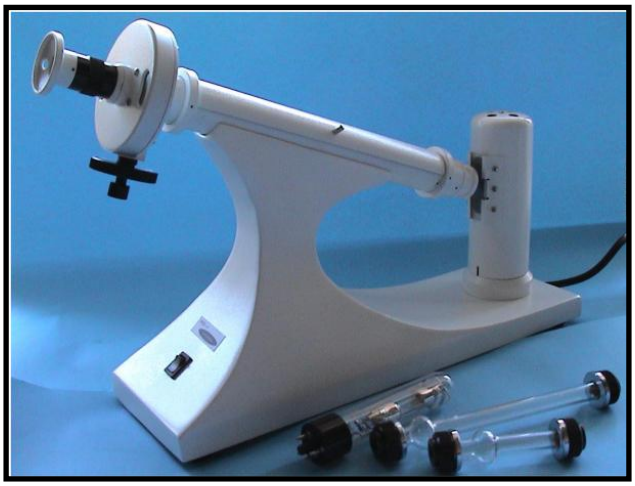
Name:	
Date:	
Course:	
Professor:	



Polarimetry of Carbohydrates

Original Text Prepared By

Monica Bottorf, Jamie Chambers, Lyle McDougall, Whitney Snider, Kristy Wahl

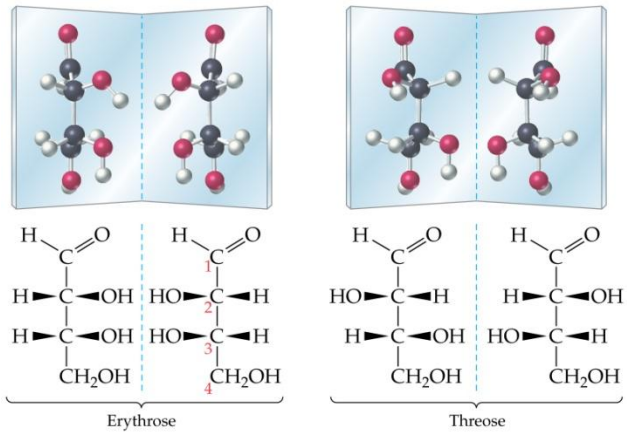
Edited FSC III

Chemistry 220 1001 Spring 2012

8 May 2012 & 23 July 2012

Polarimetry

Polarimetry is a method used to analyze the extent to which a beam of linearly polarized light is rotated during its transmission through a medium containing an optically active species. A compound is optically active if it has no plane of symmetry and is not super-imposable on its mirror image. Such compounds are referred to as being "chiral". The two molecules which make up the chiral compound are called enantiomers. Sugars and amino acids are only a few examples of substances that exhibit an optical rotary power.[1]



Erythrose and Threose exists as enantiomeric pairs. Carbon Atoms 2 and 3 are chiral. Note the mirror image relationship of hydrogen atoms and hydroxide groups.

Reproduced from http://www.bing.com/images/search?q=polarimetry++carbohydrates&view=detail&id=92C4742B53C4D7A2C4CD20AF2CF8E1C4DFE0DEE0&first=0&FORM=IDFRIR, accessed 7 May 2012, 1232 hours PDT.

Chiral molecules have an asymmetrical center which responds to light as a lens and rotates the plane of the light. A simple way to remember this “asymmetry” is that the last carbon of the molecule that has 4 different “R” groups is asymmetrical; if there are two or more identical “R” groups on that carbon atom, then the atoms are said to be symmetrical.

Enantiomeric molecules rotate light by exactly the same amount but in the opposite direction.[2] Enantiomers are isomers that are non-superimposable, e.g., your hands are enantiomers: when held up to each other, they are mirror images, yet if you attempt to superimpose them on each other, the thumbs and pinkies are out of alignment.

The degree to which a substance rotates light may be used to determine:

- The identity of the substance.
- The optical purity or enantiomeric excess of the substance.
- The concentration of a known substance in a solution.

Polarimetry is used consistently in quality control within the pharmaceutical industry, the flavor and food industry, the fragrance and essential oil industry, as well as the chemical industry. The optical purity of the product can be determined by measuring the specific rotation of compounds such as amino acids, antibiotics, steroids, vitamins, lemon oil, various sugars, and polymers and comparing them with the reference value (if the specific rotation of the pure enantiomer is known).[3]

A polarimeter (figure upper right) is an insatrument that allows one to determine light rotation through a sample in a specific direction. The light from the light source (Na lamp) passes through the polarizer to become plane polarized (figure at lower right). While ordinary light has waves emanating in every direction, plane polarized light moves parallel to a single plane. This is significant as the plane of polarized light is what allows us to see the optical activity of the substance. The optical activity of the substance will actually rotate the plane of polarized light. If the polarized light rotates in a clockwise manner, the substance is in the D form of configuration. If the light rotates counterclockwise, it is in the L form of configuration. Once the configuration of a substance is known, you can determine several important factors about that substance. For instance, certain enzymes will only bind with molecules in the D form while others will only bind with those in the L form. Knowing the configuration of a substance will allow you to determine whether or not it will fit into the active site of a certain enzyme.[4]

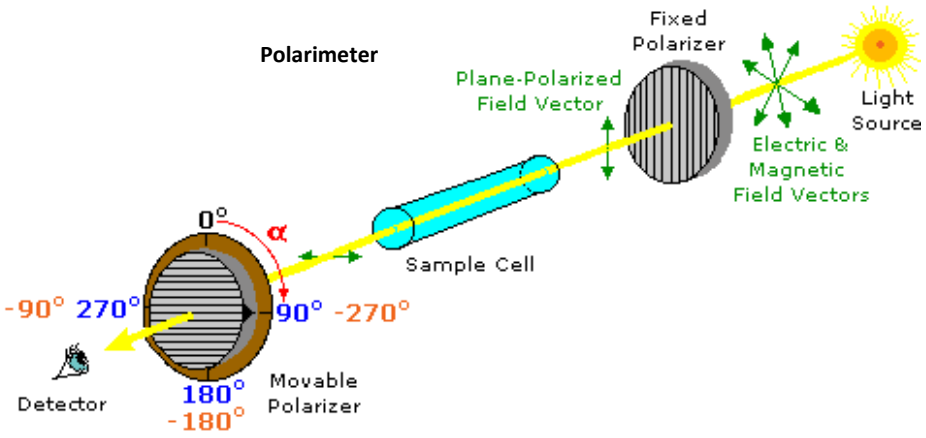


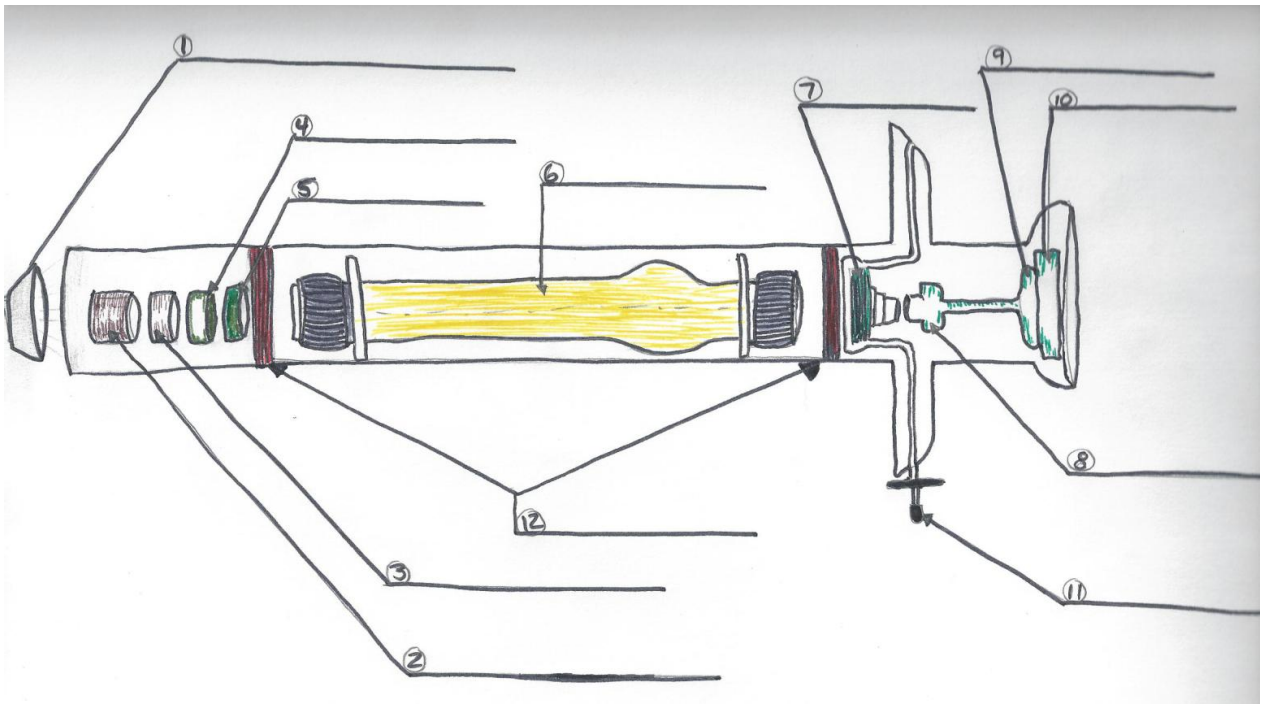
Figure Reproduced from
http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/sterism2.htm,

Mechanical Make-up of the Polarimeter

1. Light Source **2. Collector Lens** **3. Color Filter** **4. Polarizer** **5. Half-Wave Plate**

6. Test Tube **7. Polarization analyzer** **8. Object lens** **9. Eye Lens**

10 Magnifying Glass **11. Dial Rotary Hand Wheel** **12. Protective Plate**



The polarimeter measures the optical rotation which is then used to determine the specific rotation. The specific rotation of a chemical compound is defined as the observed angle of optical rotation when plane-polarized light is passed through a sample with a path length of 1 decimeter. A negative value indicates L rotation while a positive value indicates D rotation.[5] The specific rotation is given by the following equation:

$$[\alpha]_T^{\lambda} = \frac{(\text{Observed Rotation}) \bullet (100)}{(\text{Solution Height})(\text{Mass of Substance})}$$

$$[\alpha]_T^{\lambda} = \text{specific rotation} : [\alpha] \text{ at some wavelength},$$
$$\lambda \text{ (usually from Na), and at some temperature, T (}^{\circ}\text{C)}$$

Based on the results obtained from conducting the following experiment, one may use this equation to determine the specific rotations of an assigned sugar.

Experimental

Polarimetry: Rotation of Carbohydrates

The purpose of this experiment is to learn how polarimetry works by determining the specific rotation of a sugar; the extent to which different carbohydrates rotate light,[6] and the identity of the sugar used in the experiment. Utilizing a polarimeter, you will record multiple specific rotations of the samples and use the average to calculate the final specific rotation. Once you have the final specific rotation of the unknown sugar, use the following table to determine its identity:

Possible Unknown Sugars

Name (Common Names)	Specific Rotation [α] (°)
D – Fructose (D – Levulose)	-86
D – Glucose	+98
D – Galactose	+82
D – Allose	+15
Sucrose	+64.5
Maltose	+118
Reproduced from: http://www.xula.edu/chemistry/documents/orgleclab/Stereochemistry_procedure_v2.pdf, accessed 6 May 2012, 1530 hours PDT.	

Materials

Chemicals	Equipment
	Spatula
Unknown Sugar, 5 grams in 100 mL of distilled water	4 - 100 mL Beakers
	Graduated Cylinder
	Glass Stirring Rod
	Polarimeter

Polarimetry Method

Turn on the polarimeter: the sodium lamp requires a minimum 10 minute warm up period prior to use. If you watch the initial start-up of the lamp, you'll observe multiple colors as the sodium is ignited in the vacuum tube. It will eventually settle down to the 589 nm yellow color.

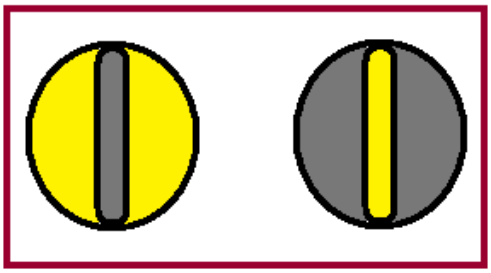
Prepare the sample solutions as directed while waiting for the discharge tube to warm up. Record your sample preparation data carefully in the data table.

After the minimum 10 minute warm-up period, obtain one of the polarimeter sample tubes. Remove one lens assembly from one end of the sample tube and inspect it for cleanliness. If the sample tube is clean, rinse it with water (distilled is preferred, however, tap will work). Cautiously shake what left-over water you can out of the sample tube. Just like lining a buret with titrant prior to adding the titrant to a washed buret, rinse the sample tube with a minimum of your test sample, dump it out, then fill the tube with test sample as follows.

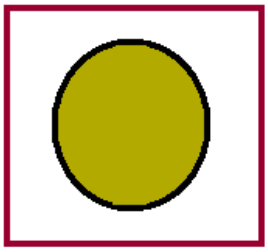
Pour one of your prepared samples into the sample tube (with this polarimeter, there are two sample tubes: a 1 dm and a 2 dm tube – use whichever you deem the most appropriate for your available sample volume), remembering to expel any air bubbles that may develop as best as possible (you won't get them all out, so just do the best you can). Remaining air bubbles will float to the top of the sample tube bulge so as to not interfere with the reading[s]. Wipe off the opening of the tube and screw the lens assembly back onto the sample tube.

Place the sample tube into the sample tube holder and close the lid.

Look through the eyepiece – you'll have to put your eye fairly close to the eyepiece to observe one of the two images at the right. NOTE: for those of you who wear glasses, you may need to remove your glasses to view this pattern appropriately. The image at right approximates what you'll see as you look into the eyepiece at this time.



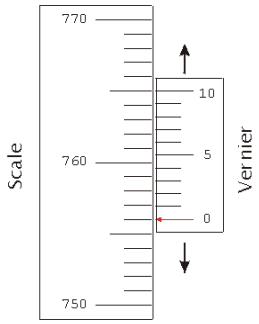
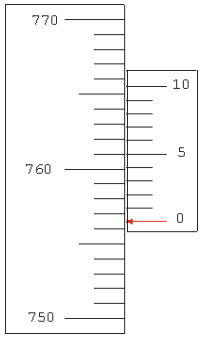
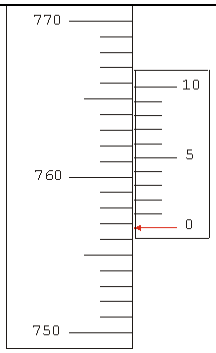
On the bottom of the polarimetry head are two small stacked wheels: one larger than the other. The larger wheel is the coarse control and the smaller is the fine control to move the dial for light rotation measurement.



Rotate the larger dial so that the caliper scale on the right side of the polarimeter (viewed through the magnifying lens just to the right of the eye-hole) moves up the scale until you observe the image in the viewing field as shown at right.

When you have obtained (are viewing) the image at the top of this page in the viewing field, record the observed rotation by reading it off the vernier scale. The vernier scale on the polarimeter is read through the magnifying lens at the right (or left) of the eyepiece.

The caliper scale can be tricky to read if you've never read one before. Click here for a really great helper link to reading this sort of scale. A portion of the information from the link is presented/reproduced/modified below to effect your comprehension of caliper reading more readily.

		
A Vernier allows a precise reading of some value. In the figure, above, the Vernier moves up and down to measure a position on the Scale. *The ["**red line**]" is the line on the vernier labeled "0". Thus the measured position is almost exactly 756 in whatever units the scale is calibrated in.*	*If we do another reading with the vernier at a different position, the pointer (the [**red line**] marked 0) may not line up exactly with one of the lines on the scale. Here the "pointer" lines up at approximately 756.5 on the scale.* *If you look, you will see that only one line on the vernier lines up exactly with one of the lines on the scale, the 5 line. This means that our first guess was correct: the reading is 756.5.*	*The [**red line**] points to a value that is obviously greater than 756.5 and also less than 757.0. Looking for divisions on the vernier that match a division on the scale, the 7 line matches fairly closely. So the reading is about 756.7.* *In fact, the 7 line on the vernier appears to be a little bit above the corresponding line on the scale. The 8 line on the vernier is clearly somewhat below the corresponding line of the scale. So with sharp eyes one might report this reading as 756.73.*
Reproduced in part and modified from http://www.upscale.utoronto.ca/PVB/Harrison/Vernier/Vernier.html, accessed 4 May 2012, 0944 hours PDT.		

The vernier on the polarimeter is read in the same manner. Do note that polarimetry experiments are temperature sensitive, so obtain your sample reading with efficiency and effectiveness and move on to the next sample.

Prior to working with the next sample, though, empty out your current sample from the sample tube into the sink with running water, rinse it with water, cautiously shake out what water you can and repeat the lining and filling process to obtain your next data.

It is important to note that temperature plays a significant role in this experiment and has the potential to impact specific rotation. This is due to the polarimeter itself and the amount of heat that it gives off, which is anywhere from approximately -17.5° C to -16.6° C. As the experiment progresses, the amount of heat that the polarimeter radiates will increase and therefore have the potential to change the physical properties of the solutions. It is for this reason that the amount of samples processed in this experiment is limited to three (i.e., read the same sample three times). Samples should be processed expeditiously and with the utmost care and efficiency. Doing so will ensure you are able to collect the most accurate data.

When you have completed your data collection, empty out the sample tube, remove BOTH sets of lens assemblies carefully and rinse the whole apparatus with water. Dry the equipment off as best as possible and return it to the Styrofoam packing for drying and storage.

Place the dust cover over the polarimeter when the experiment is completed.

Work up your data as indicated in the experimental write up.

Side View of Polarimeter

Data Collection

Sugar Data Recording

Sugar:	
Solvent:	
Mass of Carbohydrate:	
Initial Volume of Solution:	
Final Volume of Solution:	

Rotation 1	
Rotation 2	
Rotation 3	

Record the overall average for Sample 1: _____

Record the height (in cm) of the solution: _____

Calculate the specific rotation using the following formula:

$$[\alpha]_T^{\lambda} = \frac{(Observed\ Rotation) \bullet (100)}{(Solution\ Height)(\text{Grams/100 mL Amino Acid})}$$

Record the Specific Rotation of the Unknown Sugar: _____

Identify the Sugar: _____

Sources

1. Hamilton, B. [Internet]. 20.2 polarimetry properties and terminology. [updated 2011 November 23; cited 2012 May 7]. Available from: http://stason.org/TULARC/science-engineering/chemistry/20-2-Polarimetry-properties-and-terminology.html

2. Martin, Gròf I. [Internet]. Polarimetry. [cited 2012 May 6]. Available from: http://www.fpharm.uniba.sk/fileadmin/user_upload/english/Department_selection/Dep._of_Physical_Chemistry_of_Drugs/Physics/POLARIMETRY.pdf

3. UCLA Chemistry and Biochemistry [Internet]. Polarimetry. [updated 2011 August 31; cited 2012 May 6]. Available from:

   http://www.chem.ucla.edu/~bacher/General/30BL/tips/Polarimetry.html

4. Carman, Franklin S., III., Ph.D. [Internet]. CHEM 220. Biological chemistry: introduction to carbohydrates and carbohydrate metabolism. [cited 2012 May 7]. Available from: http://www.drcarman.info/

5. Eduweblabs.com. [Internet]. Specific rotation. [cited 2012 May 7]. Available from: http://eduweblabs.com/Database/Lab_FoldersP/SRotation/SRotation_P.html

6. Glas-Col, Ltd. [Internet]. Polarimeters in college chemistry courses. [cited 2012 May 6]. Available from: www.glascol.com/supportdoc/download/id/32

Questions

Lyle McDougall, Jamie Chambers, Kristy Wahl, Monica Bottorf, Whitney Snider

1) What configuration was the sugar in? Explain how you were able to determine this.

2) What is an enantiomer?

3) Describe how you determined the identity of the unknown sugar.

4) What is polarimetery?

5) What is a chiral carbon? Draw four D-carbohydrates (in Hayworth projections) using lectures, the internet, or some other source.

6) What kind of glycoside bonds does sucrose have? Provide an illustration for this.

7) What kind of carbohydrates do humans metabolize?